STEROID TRANSFORMATIONS

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY
TO THE
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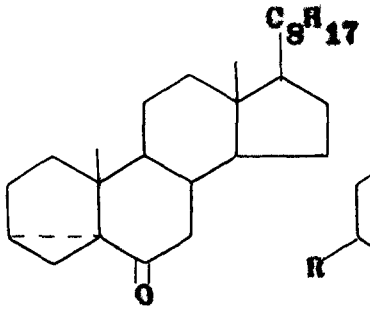
RESUME

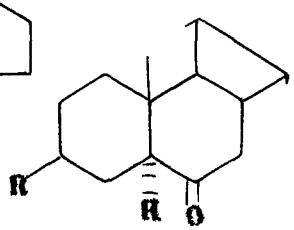
PART - I

Baeyer-Villiger oxidation of steroidal ketones

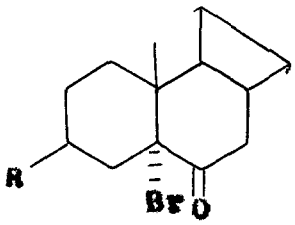
Peracid oxidation of steroidal ketones, saturated and unsaturated as well, has been a prominent part of research in these laboratories for a number of years. The substrates employed in these studies were 3α,5-cyclo-5α-cholestan-6-one (I), its 3β-halo derivatives (II-IV), 5-bromo-5α-cholestan-6-one (V), its 3β-acetoxy analogue (VI), cholest-5-en-7-one (VII), its 3β-acetoxy analogue (VIII), cholesta-3,5-dien-7-one (IX), cholesta-2,4-dien-6-one (X), 6β-bromocholest-4-en-3-one (XI), 4α-acetoxycholest-5-en-3-one (XII), methyl 5-keto-5,6-secocholestan-6-oate (XIII), methyl 5-keto-5,6-secocholest-3-en-6-oate (XIV) and methyl 5-keto-4,5-secocholestan-4-oate (XV).



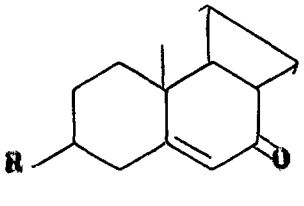
(I)



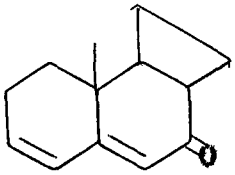
(II) R, Cl
(III) R, Br
(IV) R, I



(V) R, H
(VI) R, OAc



(VII) R, H
(VIII) R, OAc



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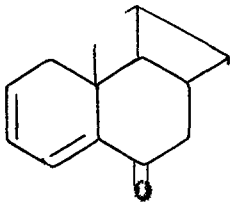

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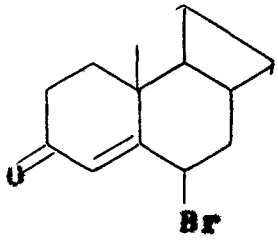

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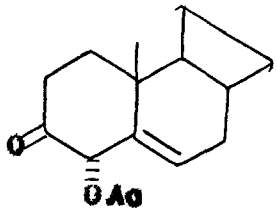

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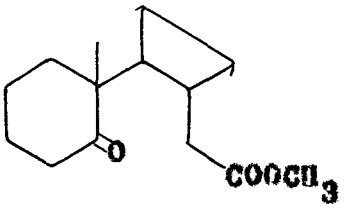

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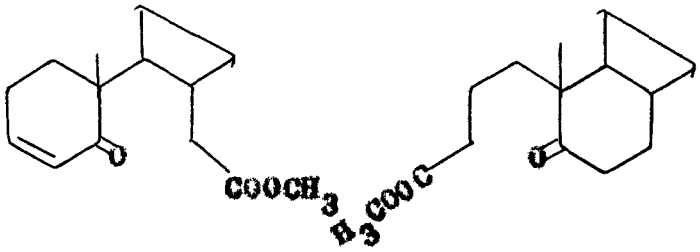
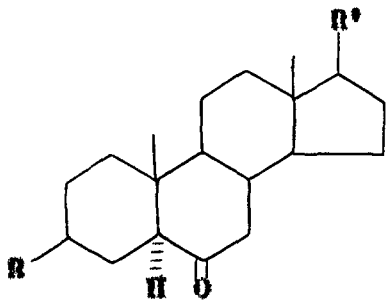
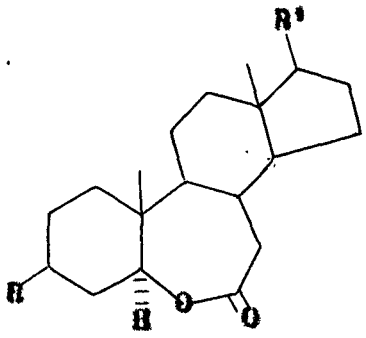


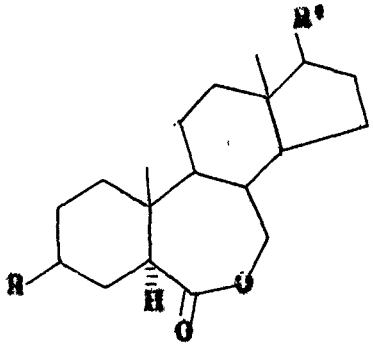
(XIV) (XV)

A. Saturated ketones

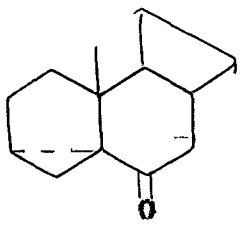
In an attempt to extend oxidation studies to hitherto unexplored ketones of the β-sitostane and cholestane series we examined the perbenzoic acid oxidation of 6-oxo-5α-β-sitostanyl acetate (XVI) and 5α-cholestane-3,6-dione (XVII). Peracid oxidation of (XVI) provided the expected 6-oxa lactone (XVIII) as well as its 7-oxa isomer (XIX). This observation contrasted with the earlier study of oxidation of 3β-acetoxy-5α-cholestan-6-one (XX) and 5α-cholestan-6-one (XXI), reported by Fonken and Wiles to have stereospecifically provided only the 6-oxa isomers (XXII) and (XXIII), respectively through superior migratory aptitude of tertiary C5 relative to secondary C7. This discrepancy prompted us to reexamine the peracid oxidation of (XX), (XXI), (I-III) and 3β-hydroxy-5α-cholestan-6-one (XXIV).



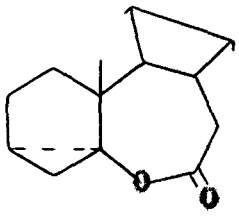




	R	R'		R	R'		R	R'
(XVI)	AcO	$C_{10}H_{21}$	(XVIII)	AcO	$C_{10}H_{21}$	(XIX)	AcO	$C_{10}H_{21}$
(XX)	AcO	C_8H_{17}	(XXII)	AcO	C_8H_{17}	(XXV)	AcO	C_8H_{17}
(XVI)	H	C_8H_{17}	(XXIII)	H	C_8H_{17}	(XXVI)	H	C_8H_{17}
(II)	Cl	C_8H_{17}	(XXVII)	Cl	C_8H_{17}	(XXVIII)	Cl	C_8H_{17}
(III)	Br	C_8H_{17}	(XXIX)	Br	C_8H_{17}	(XXX)	Br	C_8H_{17}
(XXIV)	OH	C_8H_{17}	(XXXI)	OH	C_8H_{17}	(XXXII)	OH	C_8H_{17}

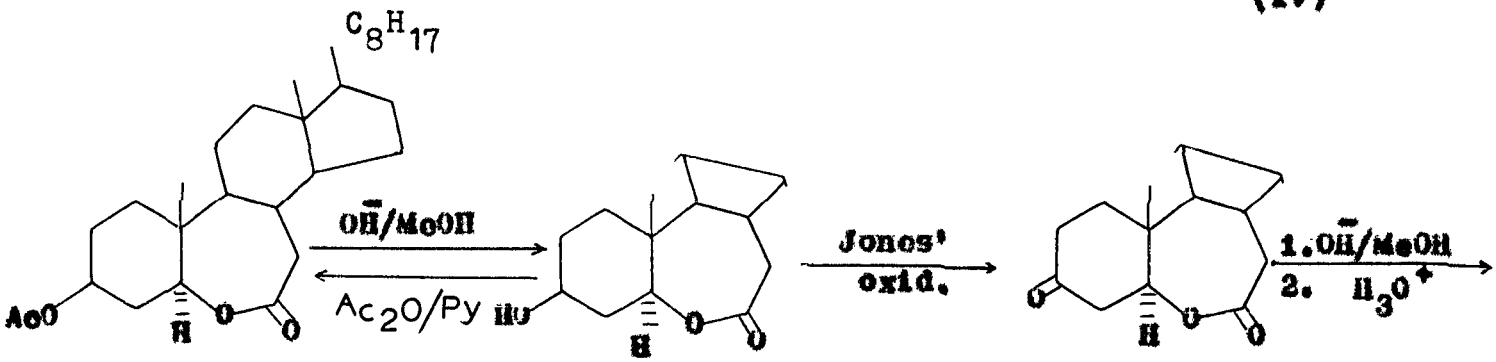


(I)

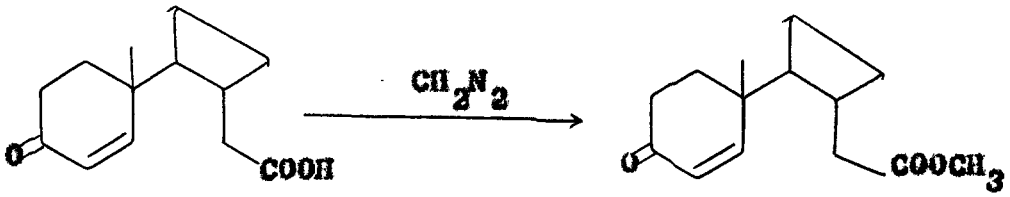


(XXXIII)

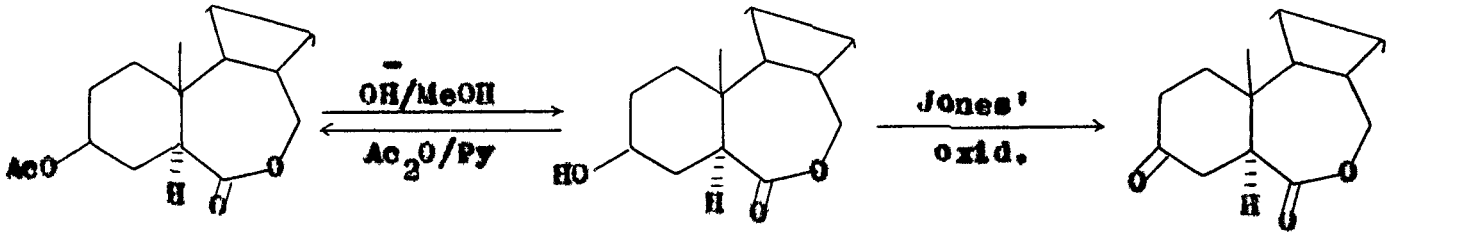
It was noted that in each case, both isomers were obtained. The formation of 7-oxa isomer increased with the increasing bulk of C3-substituent as evidenced by the ratio of products obtained. The exception was the cycloketone (I) which furnished the 6-oxa lactone (XXXIII) alone. The structures were unequivocally established on the basis of spectral characteristics and chemical transformations, some of which are outlined below.



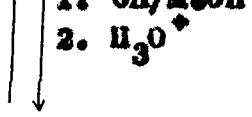
(XXII) (XXXI) (XXXIV)

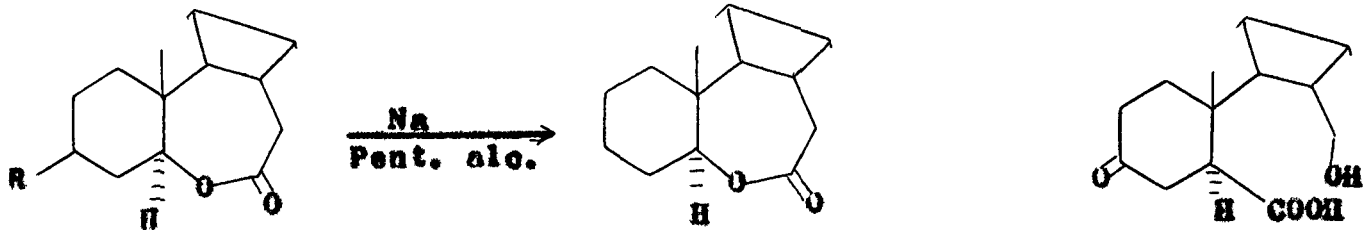


(XXXV) (XXXVI)



(XXV) (XXXII) (XXXVII)

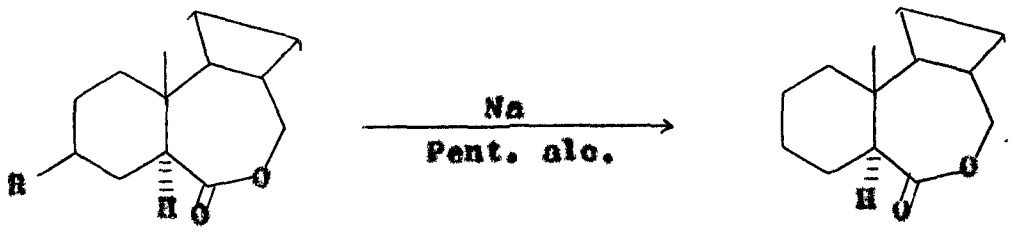




(XXVII) R,Cl
(XXIX) R,Br

(XXIII) (XXXVIII)

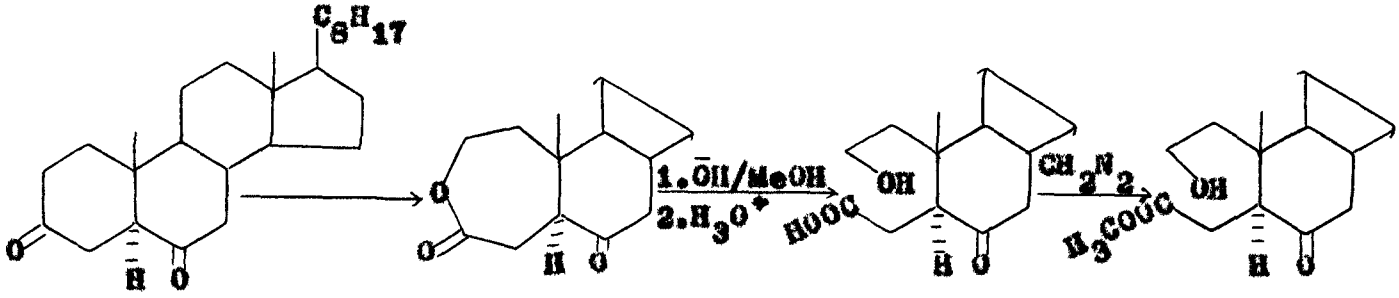
(v)



(XXVIII) R, Cl (XXVI)
(XXX) R, Br

In view of the aforementioned observations it is concluded afresh that despite being less substituted, C7 competes effectively with C5 for migration to electron deficient oxygen, moreso, in 3β-substituted steroids.

Baeyer-Villiger oxidation of 5α-cholestane-3,6-dione (XVII) provided exclusively the monolactone (XXXIX) and none of either the monolactones or dilactones conceivable. The structure was established on spectral evidence and chemical conversions illustrated below.



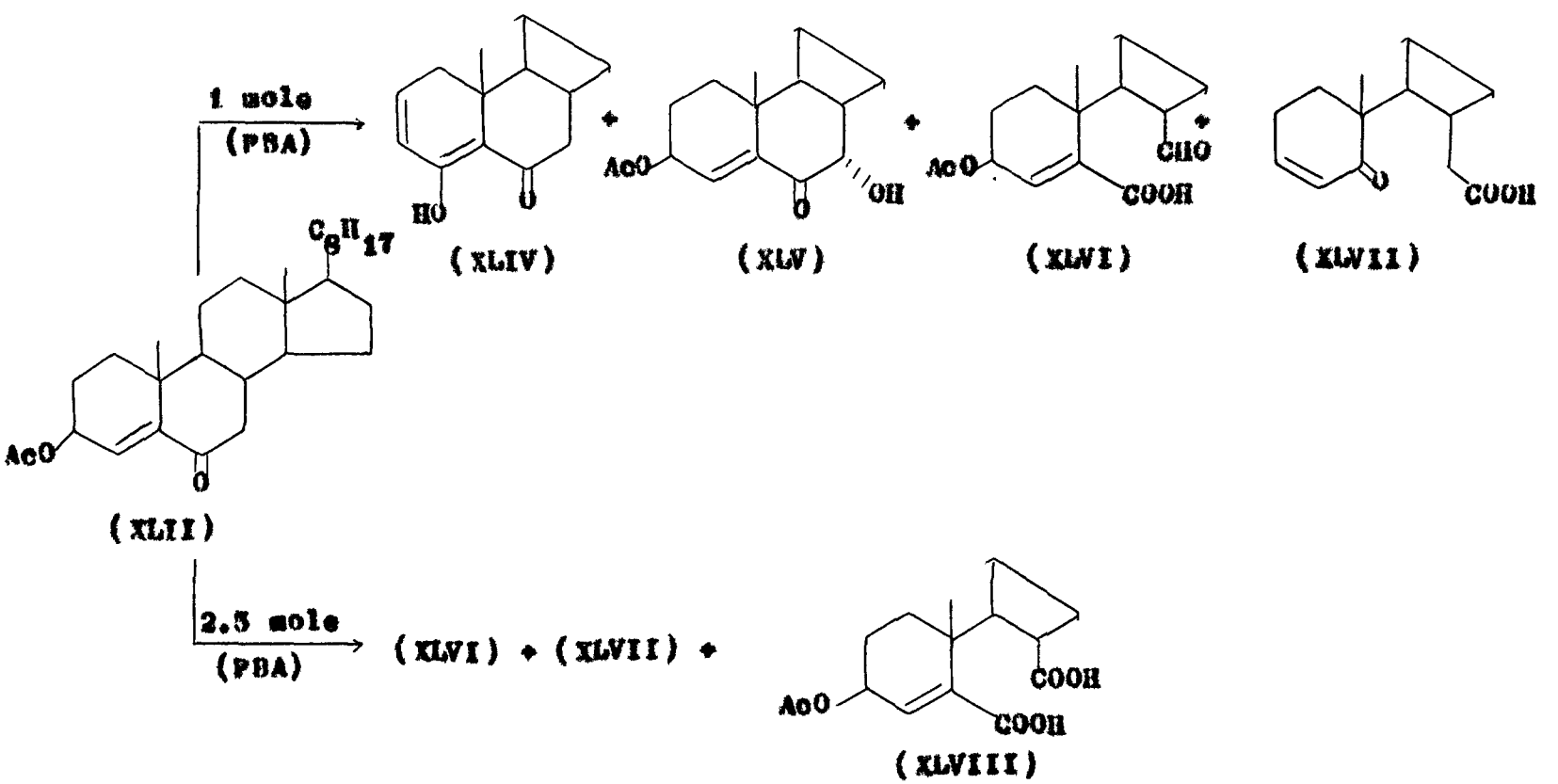
(XVII) (XXXIX) (XL) (XLI)

B. α,β-Unsaturated ketones

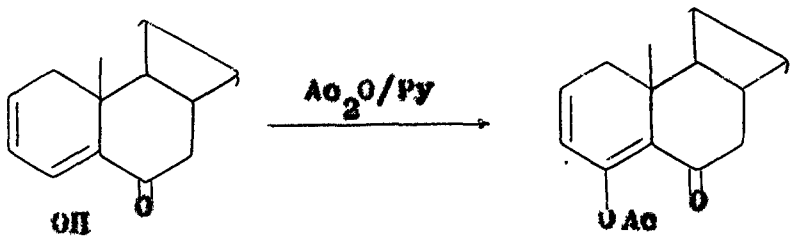
Peracid oxidation of conjugated enones is always of

interest as it furnishes a wide array of rearranged products depending upon the experimental conditions. In the hope of achieving some interesting results, 3β-acetoxycholest-4-en-6-one (XLII) and cholest-4-ene-3,6-dione (XLIII) were put to perbenzoic acid oxidations at different concentration levels. Both (XLII) and (XLIII) provided a variety of rearranged products.

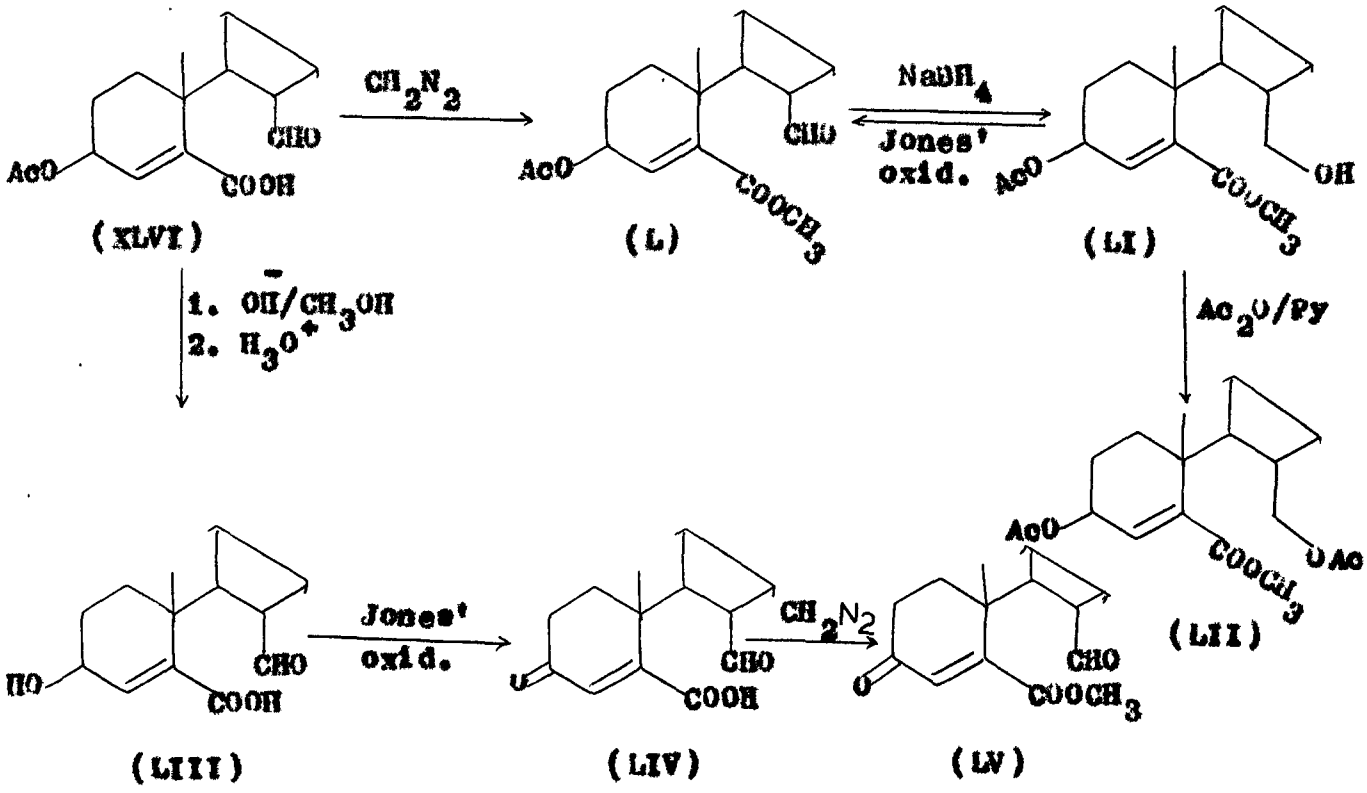
(a) The ketone (XLII) with 1 mole equivalent of perbenzoic acid gave 4-hydroxycholesta-3,4-dien-6-one (XLIV), 6-oxo-7-hydroxycholest-4-en-3β-yl acetate (XLV), 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (XLVI) and 5-oxo-5, 6-secocholest-3-en-6-oic acid (XLVII). With 2,5 mole equivalent of peracid, (XLII) provided (XLVI), (XLVII) and 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (XLVIII).



The structures were identified by spectral studies and chemical conversions.

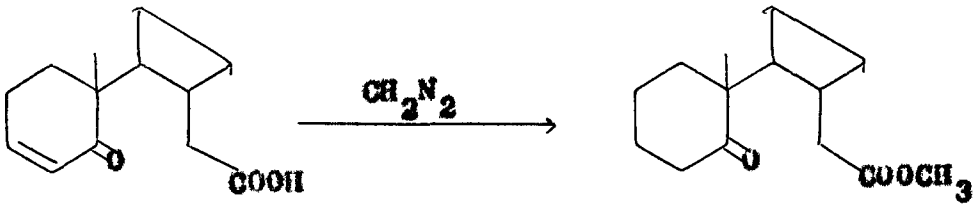


(XLIV) (XLIX)

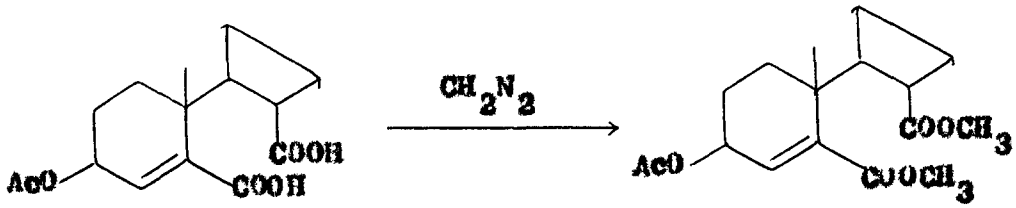


(XLVI) (L) (LI) (LII) (LIII) (LIV) (LV)

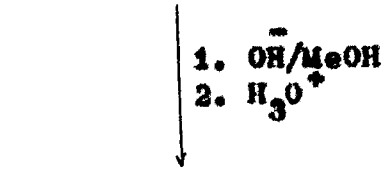
Surprisingly, the aldehydic functions in (L) and (LIV) remained unaffected during oxidation by Jones' reagent.

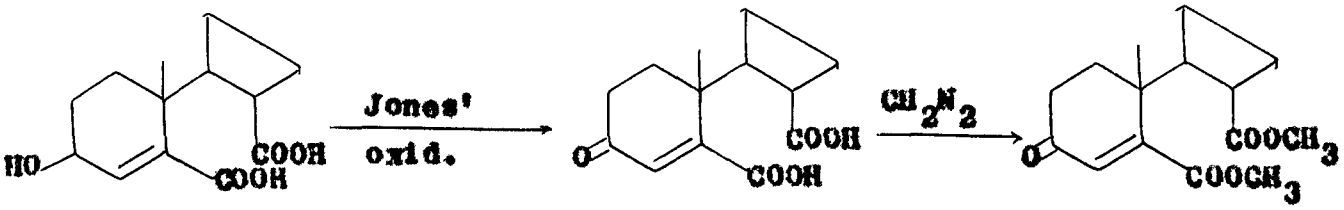


(XLVII) (LVI)

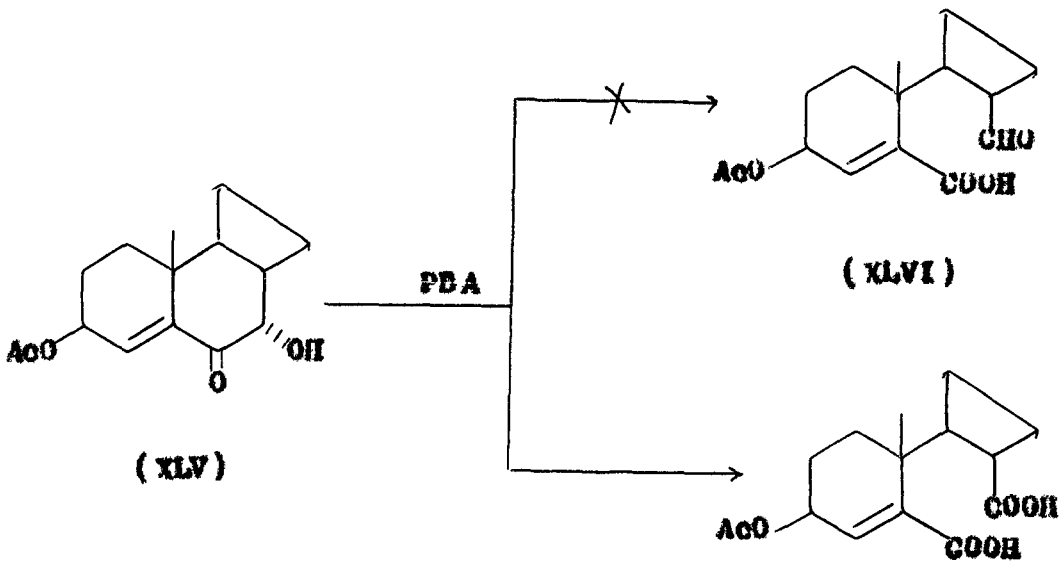


(XLVIII) (LVII)





(LVIII) (LIX) (LX)



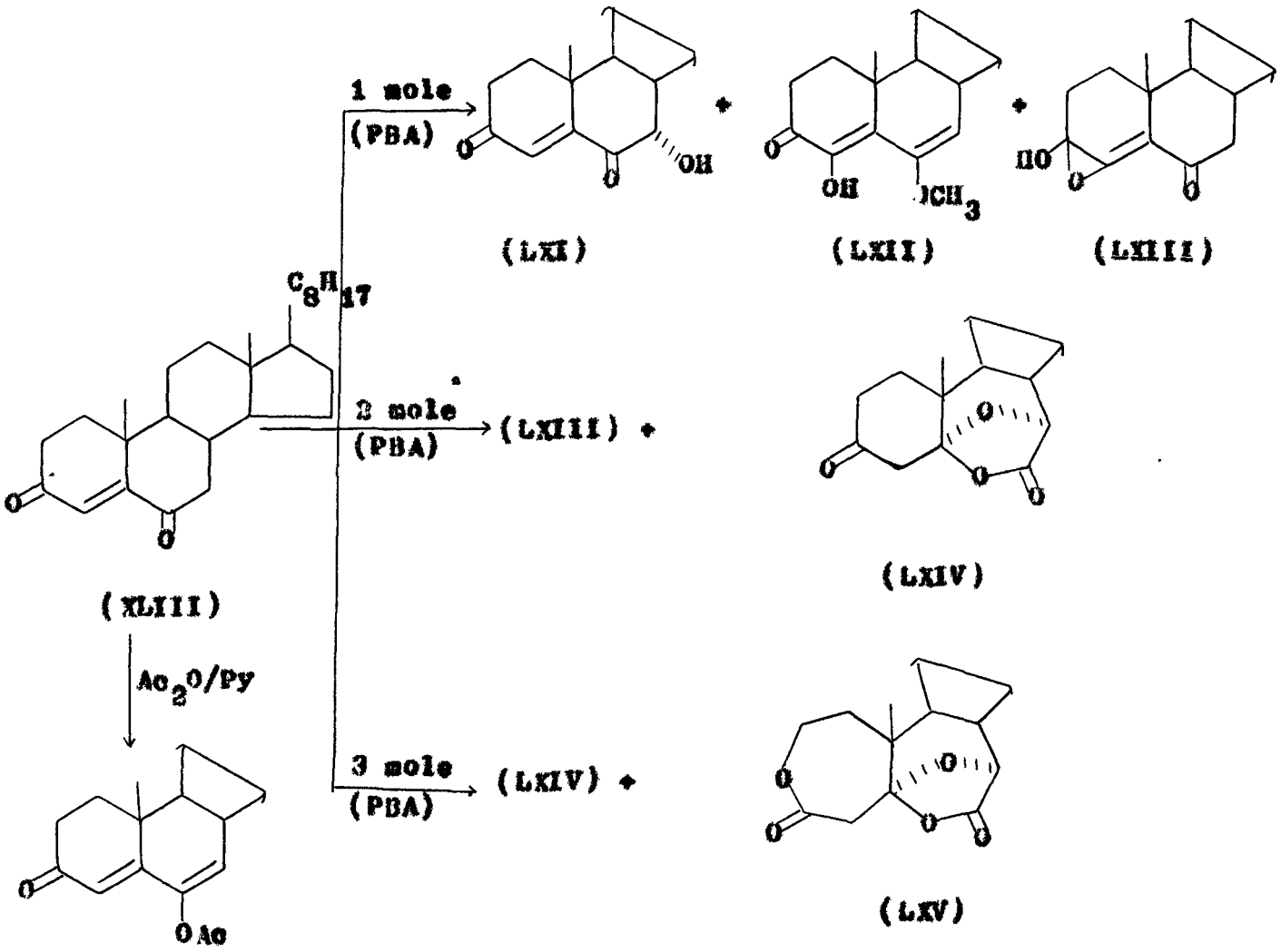
(XLV)

(XLVI)

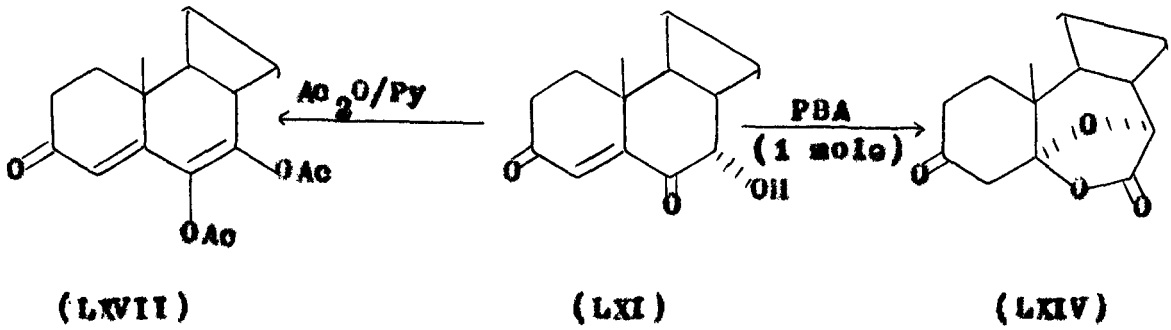
(XLVIII)

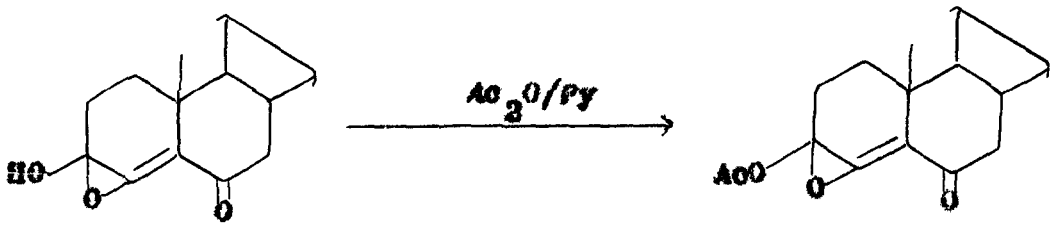
Mechanisms for the formation of the formyl acid (XLVI) and the diacid (XLVIII) have been proposed. It has been experimentally proved that (XLV) is an intermediate in the conversion of (XLII) into the diacid (XLVIII) but not to the formyl acid (XLVI). The formation of the formyl acid (XLVI) from (XLII) has been proposed to occur via an analogous pathway.

(b) Cholest-4-ene-3,6-dione (XLIII) with 1 mole equivalent of perbenzoic acid provided 7α-hydroxycholest-4-ene-3,6-dione (LXI), 4-hydroxy-6-methoxycholesta-4,6-dien-3-one (LXII) and 3-hydroxy-3,4-oxidocholest-4-en-6-one (LXIII). Reaction of (XLIII) with 2 mole equivalent gave (LXIII) and a novel product 5α,7aα-oxido-6-oxa-B-homocholestane-3,7-dione (LXIV). With 3 mole equivalent of peracid (XLIII) furnished (LXIV) and 5α,7aα-oxido-3,6-dioxa-A,B-bishomocholestane-4,7-dione (LXV).

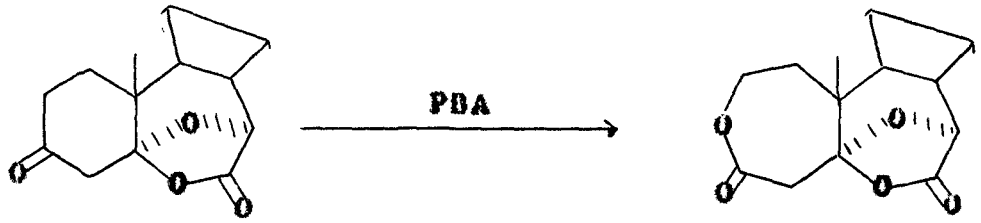


The structures were established by their physical properties and chemical transformations.





(LXIII) (LXVIII)



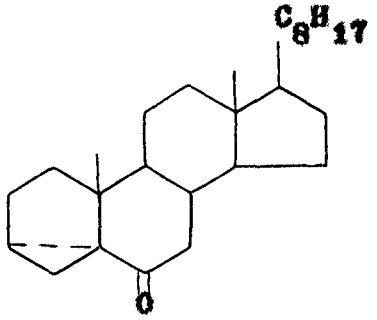
(LXIV) (LXV)

A mechanism for the conversion of (XLIII) into (LXIV) involving the intermediacy of (LXI) has been proposed.

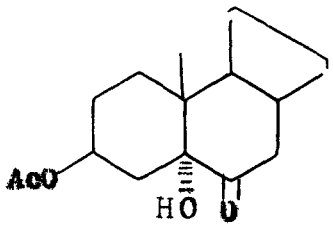
PART - II

Steroidal Tetrazoles

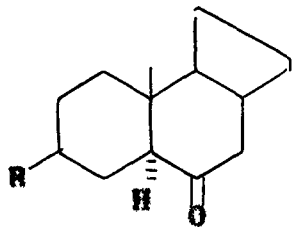
In our laboratory, we recently took to the synthesis of steroidal tetrazoles which are often claimed to be of clinical importance. Previously two 6-oxo steroids, namely 3α,5-cyclo-5α-cholestan-6-one (I) and 3β-acetoxy-5-hydroxy-5α-cholestan-6-one (LXIX) were used as substrates. However, for a systematic study, the easily accessible 5α-cholestan-6-one (XXI) and its 3β-acetoxy (XX), 3β-hydroxy (XXIV) and 3β-chloro (II) analogues were chosen. The results are summarised below.



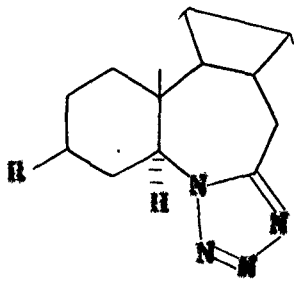
(I)



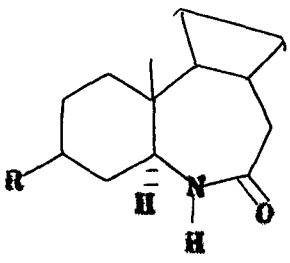
(LXIX)



(XXI) R, H
(XX) R, OAc
(XXIV) R, OH
(II) R, Cl

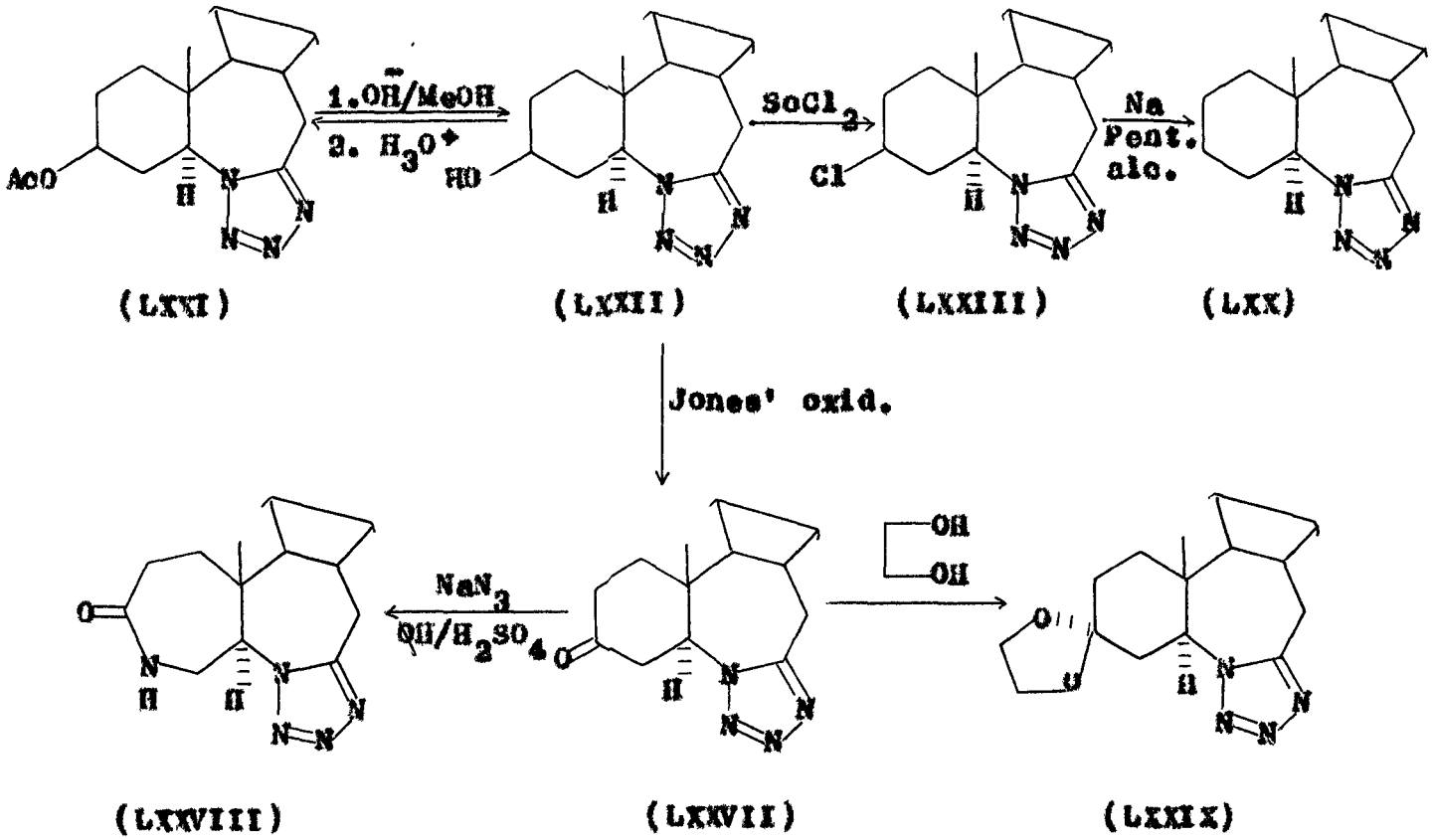


(LXX) R, H
(LXXI) R, OAc
(LXXII) R, OH
(LXXIII) R, Cl



(LXXIV) R, H
(LXXV) R, OH
(LXXVI) R, Cl

The structures were characterized by physical methods and chemical conversions as shown below.



(LXXI) (LXXII) (LXXIII) (LXX)

(LXXVIII) (LXXVII) (LXXIX)

The n.m.r. spectra of tetrazoles exhibited some noteworthy features from characterization viewpoint. There was an unusual diamagnetic shift of C13-methyl protons. Moreover, the C7a protons did not appear together as was expected of them. Attempts have been made to rationalize these facts.

PART - III

Mass Spectrometry of steroidal ϵ-lactones

A communication from this laboratory had described the mass spectra of several 6-oxa lactones (XXIII), (XXVII), (XXIX) and (XXXIII) in the cholestane series, the diagnostic feature of which was an intense peak at m/e 316. Even at that time, the limited value of this study was sounded as this peak could also result from the isomeric 7-oxa lactones. The 7-oxa lactones (XIX), (XXVI) and (XXVIII) prepared in the present study (Part-I) were subjected to mass spectral fragmentation and comparison made with the mass spectra of 6-oxa lactones. In contrast to latter, the former showed characteristic M-CH_2O peaks. This radical difference in the mass spectral behaviour of 6- and 7-oxa lactones can be made use of in distinguishing between these classes of steroidal compounds.

The following table summarises the relevant points of difference between 6- and 7-oxa lactones.

Table

	XXIII	XXVI
(1)	M-CO	M-CH_2O; no M-CO peak
(2)	m/e 318 (base peak)	m/e 318; almost negligible
(3)	m/e 262	m/e 262, 261 (almost of same intensity), 260
(4)	- - -	M-68 (loss of C3,C4,C5,C6).

These differences may be considered of diagnostic value since other isomeric lactones (XXVII and XXVIII) and (XVIII and XIX) also exhibited analogous behaviour.

Department of Chemistry
Aligarh Muslim University
Aligarh

This is to certify that the work described in this thesis is the original work of the candidate done under my supervision. The thesis is suitable for submission for the award of Ph.D. degree in Chemistry.

M. S. Ahmad

(Mohammad Shahabuddin Ahmad)
Professor of Chemistry

ACKNOWLEDGEMENT

Through these lines I wish to give an expression of my extreme gratefulness to Prof. M.S. Ahmad for his invaluable guidance and help at every stage of the work described here. He has been a constant source of inspiration to me and I owe an enormous debt to him. I am also extremely thankful to Prof. W. Rahman, Head, Department of Chemistry both for providing necessary facilities as well as for the encouragement received off and on. Dr. S.M. Osman always showed a keen interest in my progress from which I benefited.

Drs. S.M. Verma (BHU, Varanasi) and Abu Shoeb (CDRI, Lucknow) have been of tremendous help to me as regards the determination of n.m.r., i.r. and mass spectra. No praise can be too high for their sincere help. Drs. M. Mushfiq and Z.H. Chowdhry have always lent a great helping hand to me and they deserve my utmost appreciation for their honest and disinterested assistance. My other research colleagues have also been aiding me and I acknowledge that with thanks. Last but not the least, I place on record my gratitude to the CSIR (New Delhi) for financial help and Mr. I.A. Jilani for the forbearance he has shown while the manuscript was being typed.

(I.A. Khan)

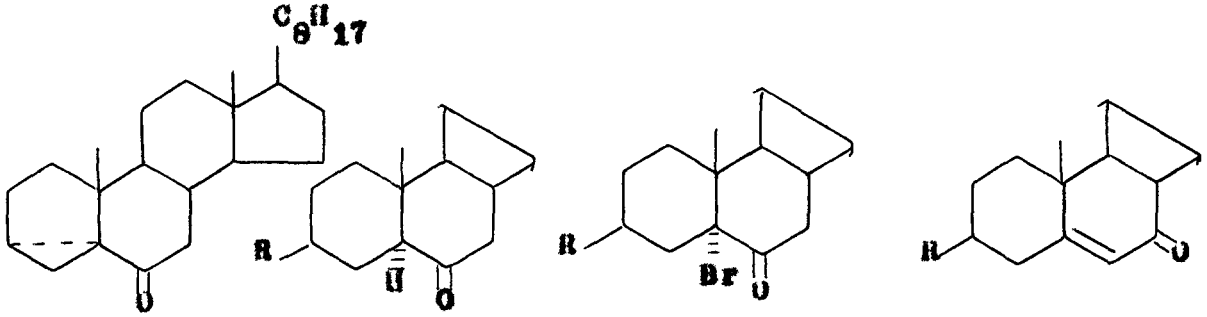
C O N T E N T S

SUMMARY

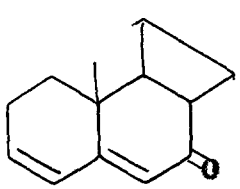
PART - I

Baeyer-Villiger oxidation of steroidal ketones

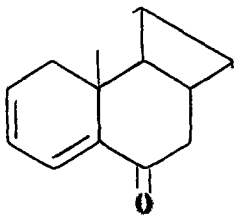
Peracid oxidation of steroidal ketones, saturated and unsaturated as well, has been a prominent part of research in these laboratories for a number of years. The substrates employed in these studies were 3α,5-cyclo-5α-cholestan-6-one (I), its 3β-halo derivatives (II-IV), 5-bromo-5α-cholestan-6-one (V), its 3β-acetoxy analogue (VI), cholest-5-en-7-one (VII), its 3β-acetoxy analogue (VIII), cholesta-3,5-dien-7-one (IX), cholesta-2,4-dien-6-one (X), 6β-bromocholest-4-en-3-one (XI), 4α-acetoxycholest-5-en-3-one (XII), methyl 5-keto-5,6-seco-cholestan-6-oate (XIII), methyl 5-keto-5,6-secocholest-3-en-6-oate (XIV) and methyl 5-keto-4,5-secocholestan-4-oate (XV).



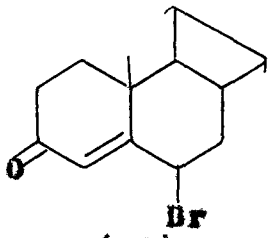
(I) (II) R, Cl (III) R, Br (IV) R, I (V) R, H (VI) R, OAc (VII) R, H (VIII) R, OAc



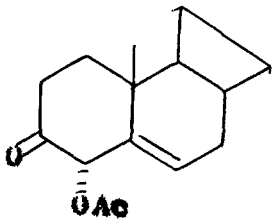
(IX)



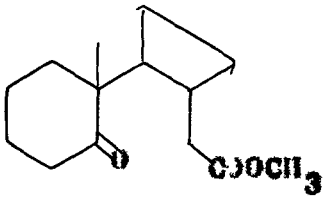
(X)



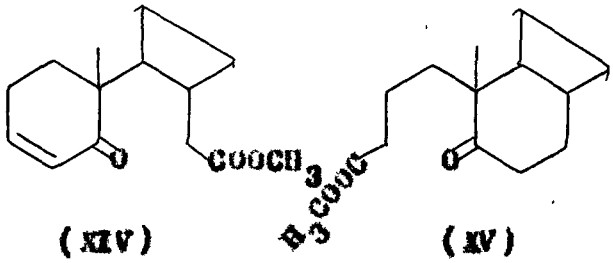
(XI)



(XII)



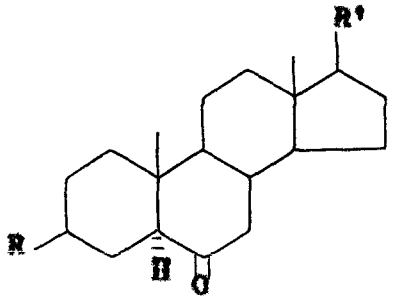
(XIII)

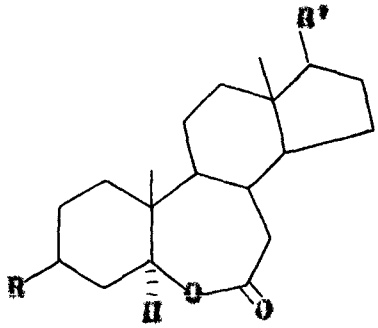


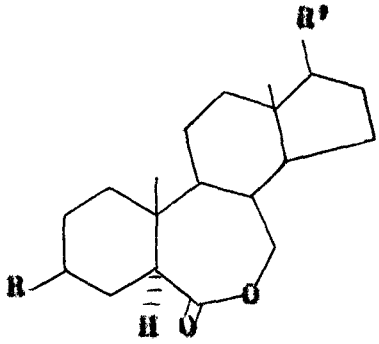
(XIV) (XV)

A. Saturated ketones

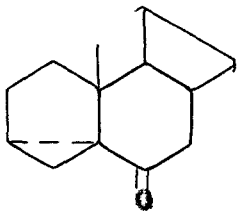
In an attempt to extend oxidation studies to hitherto unexplored ketones of the β-sitostane and cholestane series we examined the perbenzoic acid oxidation of 6-oxo-5α-β-sitostanyl acetate (XVI) and 5α-cholestane-3,6-dione (XVII). Peracid oxidation of (XVI) provided the expected 6-oxa lactone (XVIII) as well as its 7-oxa isomer (XIX). This observation contrasted with the earlier study of oxidation of 3β-acetoxy-5α-cholestan-6-one (XX) and 5α-cholestan-6-one (XXI), reported by Fonken and Miles to have stereospecifically provided only the 6-oxa isomers (XXII) and (XXIII), respectively through superior migratory aptitude of tertiary C5 relative to secondary C7. This discrepancy prompted us to reexamine the peracid oxidation of (XX), (XXI), (I-III) and 3β-hydroxy-5α-cholestan-6-one (XXIV).



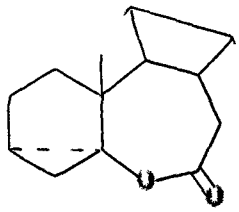




	R	R'		R	R'		R	R'
(XVI)	AcO	$C_{10}H_{21}$	(XVIII)	AcO	$C_{10}H_{21}$	(XIX)	AcO	$C_{10}H_{21}$
(XX)	AcO	C_8H_{17}	(XXII)	AcO	C_8H_{17}	(XXV)	AcO	C_8H_{17}
(XXI)	H	C_8H_{17}	(XXIII)	H	C_8H_{17}	(XXVI)	H	C_8H_{17}
(II)	Cl	C_8H_{17}	(XXVII)	Cl	C_8H_{17}	(XXVIII)	Cl	C_8H_{17}
(III)	Br	C_8H_{17}	(XXIX)	Br	C_8H_{17}	(XXX)	Br	C_8H_{17}
(XXIV)	OH	C_8H_{17}	(XXXI)	OH	C_8H_{17}	(XXXII)	OH	C_8H_{17}

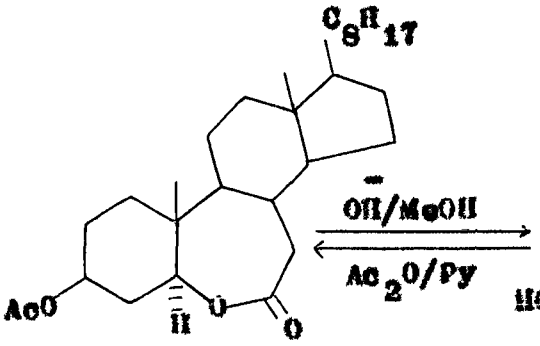


(I)

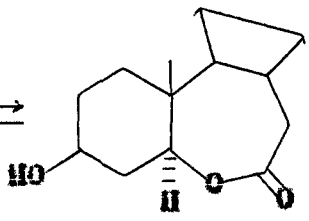


(XXXIII)

It was noted that in each case, both isomers were obtained. The formation of 7-oxa isomer increased with the increasing bulk of C3-substituent as evidenced by the ratio of products obtained. The exception was the cycloketone (I) which furnished the 6-oxa lactone (XXXIII) alone. The structures were unequivocally established on the basis of spectral characteristics and chemical transformations, some of which are outlined below.

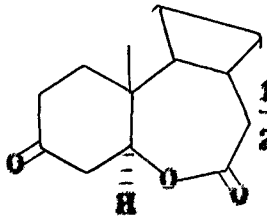


(XXII)



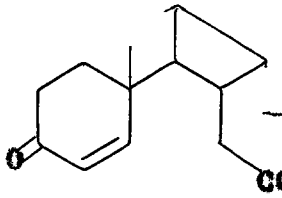
(XXXI)

Jones' oxid.

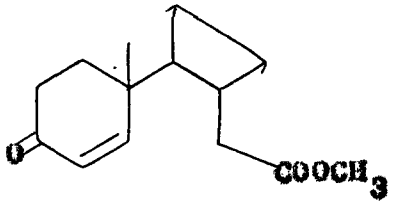


1. $\bar{O}H$/MeOH
2. H_3O^+

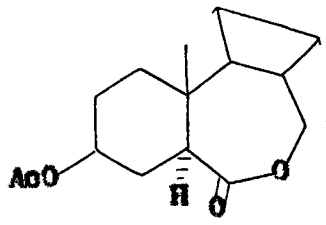
(XXXIV)



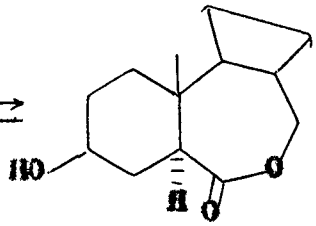
CH_2N_2



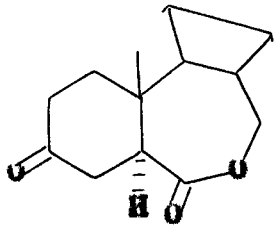
(XXXV) (XXXVI)



$\bar{O}H$/MeOH
Ac_2O/Py



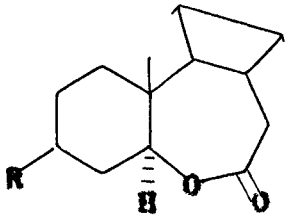
Jones' oxid.



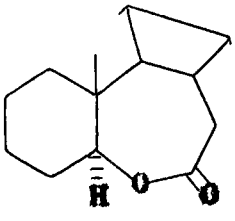
(XXV) (XXXII) (XXXVII)

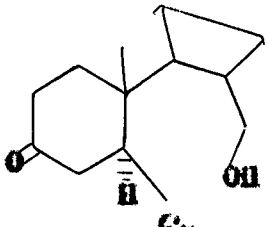
$-H_2O$

1. $\bar{O}H$/MeOH
2. H_3O^+



Na
Pent. alc.

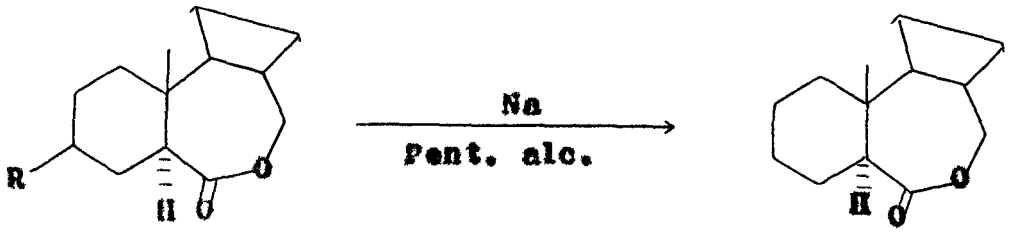




(XXVII) R,Cl
(XXIX) R,Br

(XXIII)

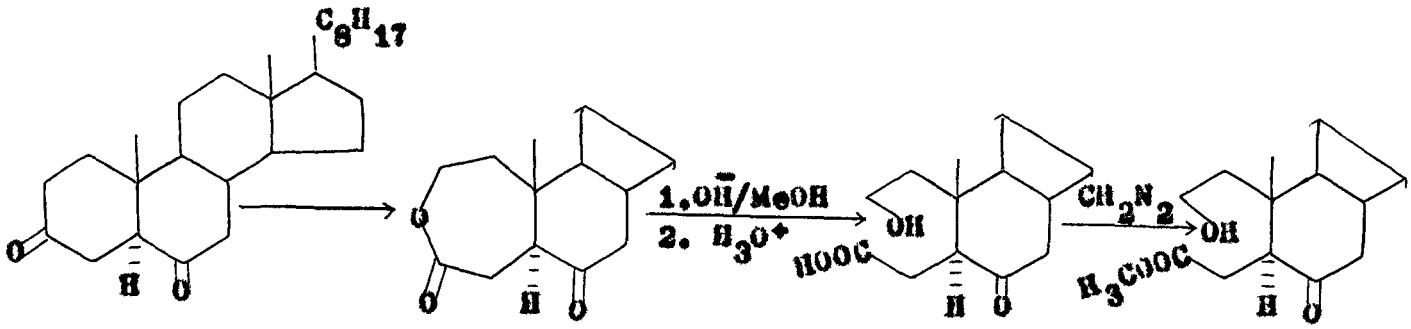
(XXXVIII)



(XXVIII) R, Cl (XXVI)
(XXX) R, Br

In view of the aforementioned observations it is concluded afresh that despite being less substituted, C7 competes effectively with C5 for migration to electron deficient oxygen, moreso, in 3β-substituted steroids.

Baeyer-Villiger oxidation of 5α-cholestane-3,6-dione (XVII) provided exclusively the monolactone (XXXIX) and none of either the monolactones or dilactones conceivable. The structure was established on spectral evidence and chemical conversions illustrated below.



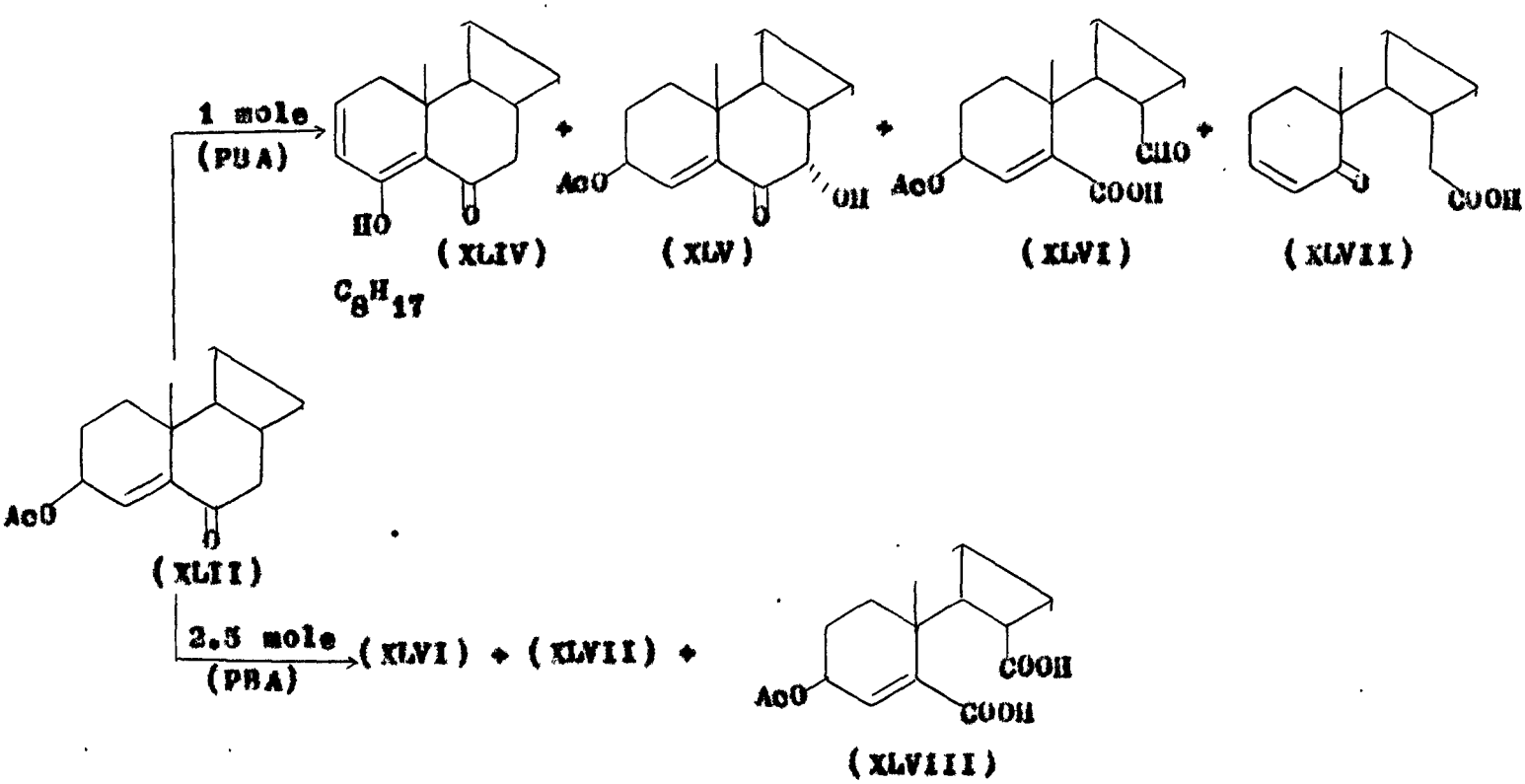
(XVII) (XXXIX) (XL) (XLI)

B. α,β-Unsaturated ketones

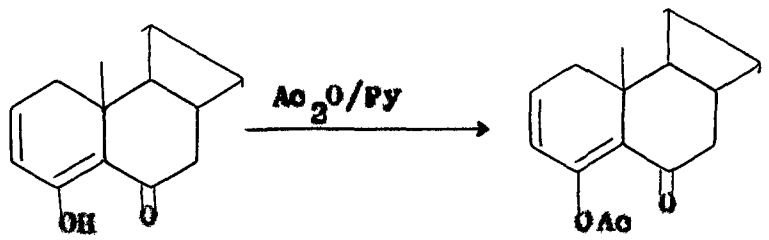
Peracid oxidation of conjugated enones is always of

interest as it furnishes a wide array of rearranged products depending upon the experimental conditions. In the hope of achieving some interesting results, 3β-acetoxycholest-4-en-6-one (XLII) and cholest-4-one-3,6-dione (XLIII) were put to perbenzoic acid oxidations at different concentration levels. Both (XLII) and (XLIII) provided a variety of rearranged products.

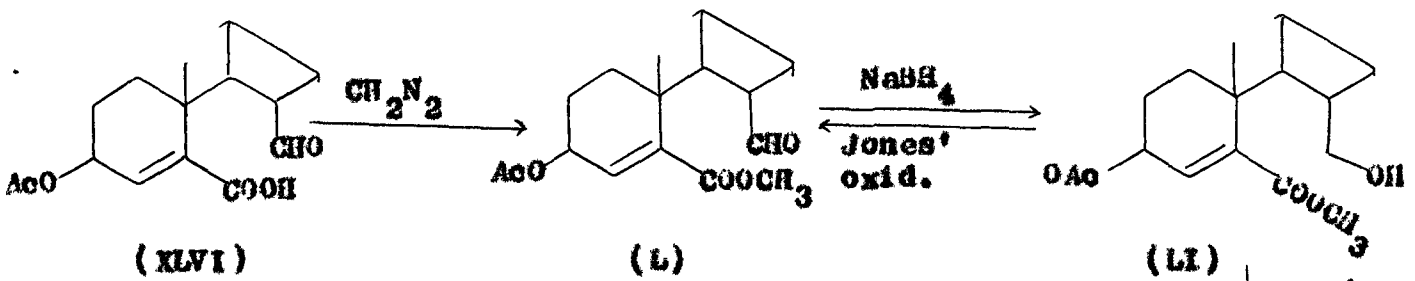
(a) The ketone (XLII) with 1 mole equivalent of perbenzoic acid gave 4-hydroxycholesta-2,4-dien-6-one (XLIV), 6-oxo-7-α-hydroxycholest-4-en-3β-yl acetate (XLV), 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (XLVI) and 5-oxo-5, 6-secocholest-3-en-6-oic acid (XLVII). With 2.5 mole equivalent of peracid, (XLII) provided (XLVI), (XLVII) and 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (XLVIII).



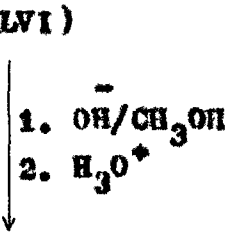
The structures were identified by spectral studies and chemical conversions.

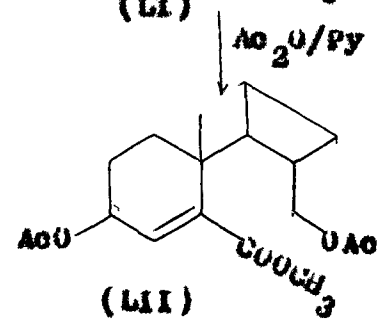


(XLIV) (XLIX)

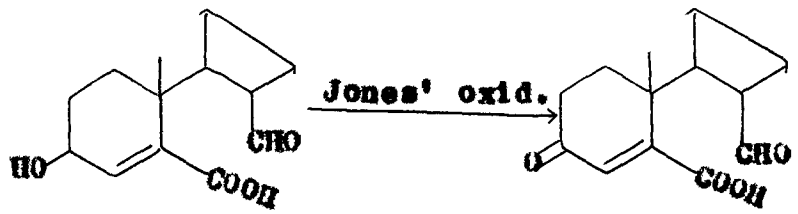


(XLVI) (L) (LI)

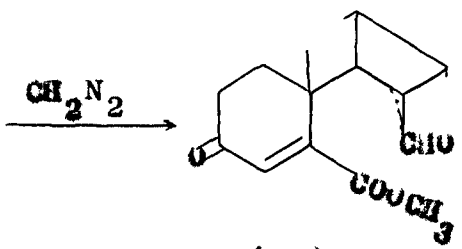




(LII)

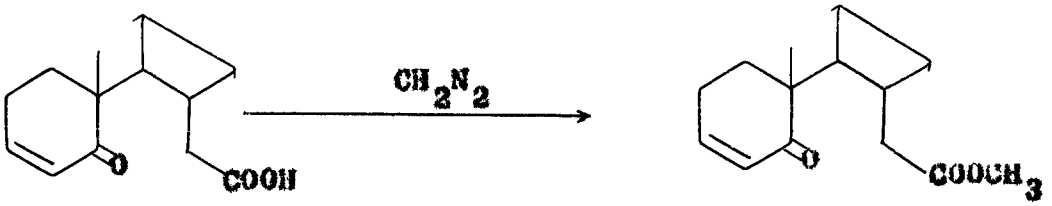


(LIII) (LIV)

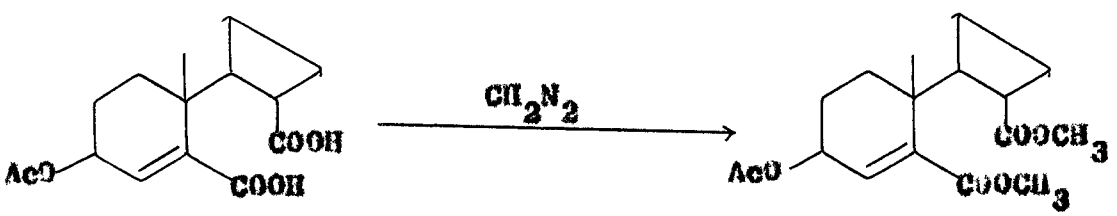


(LV)

Surprisingly, the aldehydic functions in (L) and (LIV) remained unaffected during oxidation by Jones' reagent.

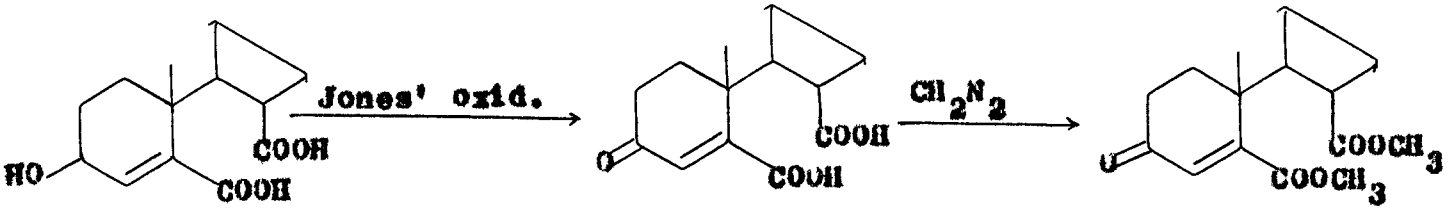


(XLVII) (LVI)

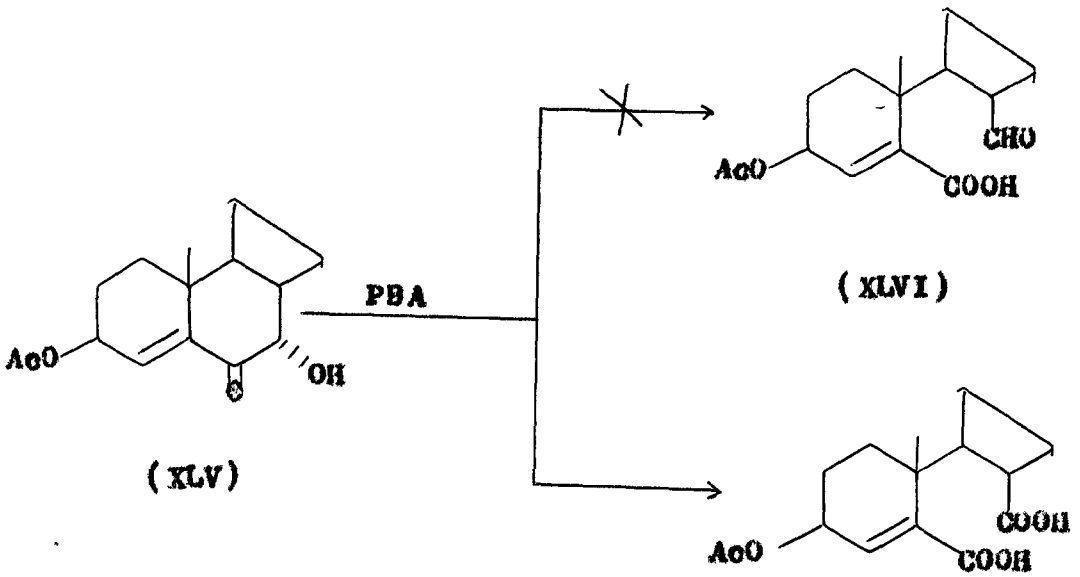


(XLVIII) (LVII)

1. $\bar{O}H$/MeOH
2. H_3O^+



(LVIII) (LIX) (LX)



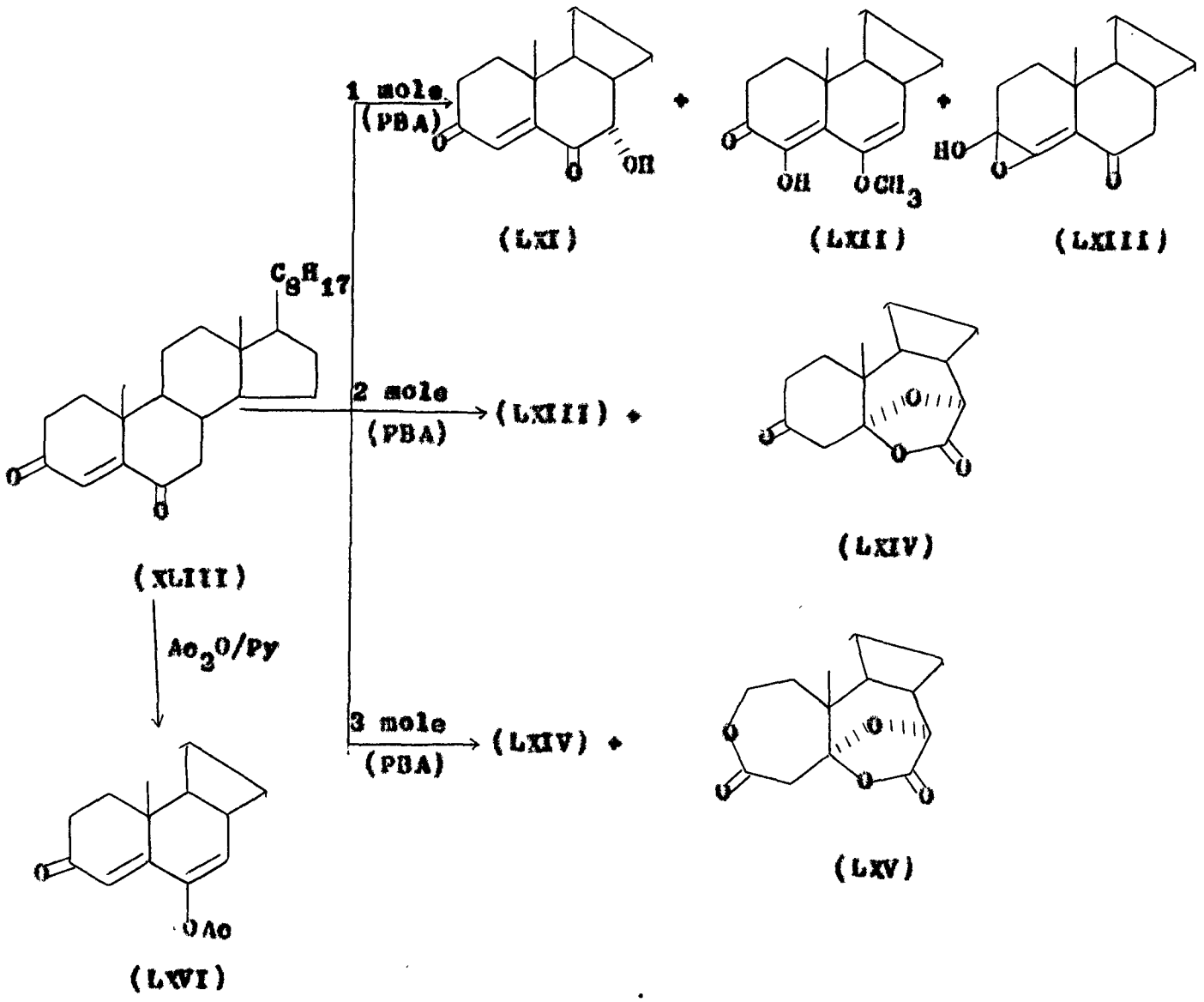
(XLV)

(XLVI)

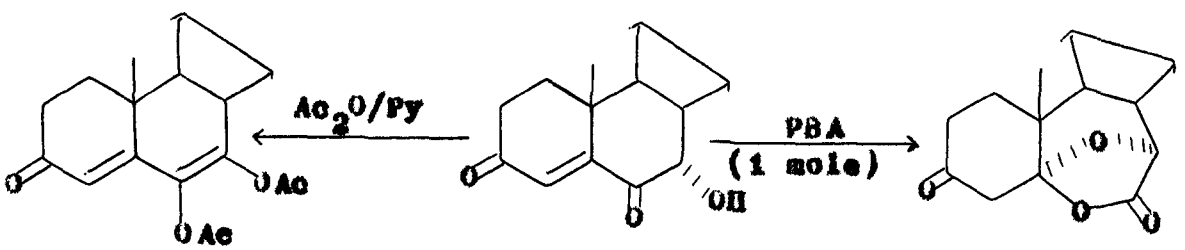
(XLVIII)

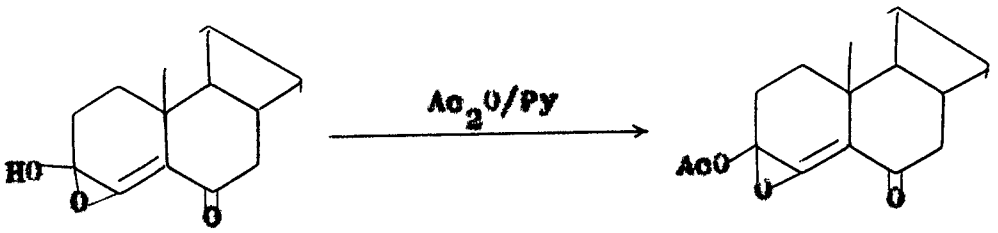
Mechanisms for the formation of the formyl acid (XLVI) and the diacid (XLVIII) have been proposed. It has been experimentally proved that (XLV) is an intermediate in the conversion of (XLII) into the diacid (XLVIII) but not to the formyl acid (XLVI). The formation of the formyl acid (XLVI) from (XLII) has been proposed to occur via an analogous pathway.

(b) Cholest-4-ene-3,6-dione (XLIII) with 1 mole equivalent of perbenzoic acid provided 7α-hydroxycholest-4-ene-3,6-dione (LXI), 4-hydroxy-6-methoxycholesta-4,6-dien-3-one (LXII) and 3-hydroxy-3,4-oxidocholest-4-en-6-one (LXIII). Reaction of (XLIII) with 2 mole equivalent gave (LXIII) and a novel product 5α,7aα-oxido-6-oxa-B-homocholestane-3,7-dione (LXIV). With 3 mole equivalent of peracid (XLIII) furnished (LXIV) and 5α,7aα-oxido-3,6-dioxa-A,B-bishomocholestane-4,7-dione (LXV).

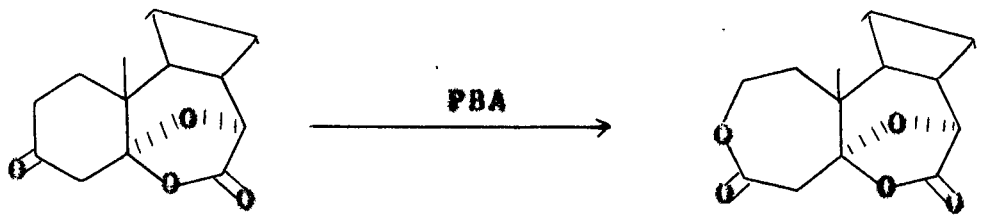


The structures were established by their physical properties and chemical transformations.





(LXIII) (LXVIII)



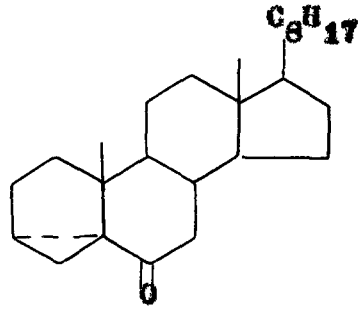
(LXIV) (LXV)

A mechanism for the conversion of (XLIII) into (LXIV) involving the intermediacy of (LXI) has been proposed.

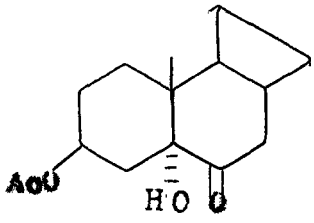
PART - II

Steroidal Tetrazoles

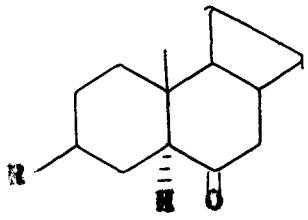
In our laboratory, we recently took to the synthesis of steroidal tetrazoles which are often claimed to be of clinical importance. Previously two 6-oxo steroids, namely 3α,5-cyclo-5α-cholestan-6-one (I) and 3β-acetoxy-5-hydroxy-5α-cholestan-6-one (LXIX) were used as substrates. However, for a systematic study, the easily accessible 5α-cholestan-6-one (XXI) and its 3β-acetoxy (XX), 3β-hydroxy (XXIV) and 3β-chloro (II) analogues were chosen. The results are summarised below.



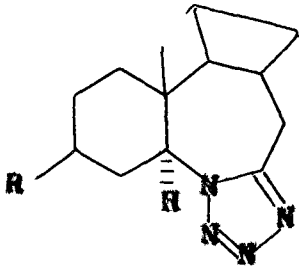
(I)



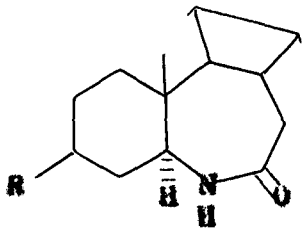
(LXIX)



(XXI) R, H
(XX) R, OAc
(XXIV) R, OH
(II) R, Cl

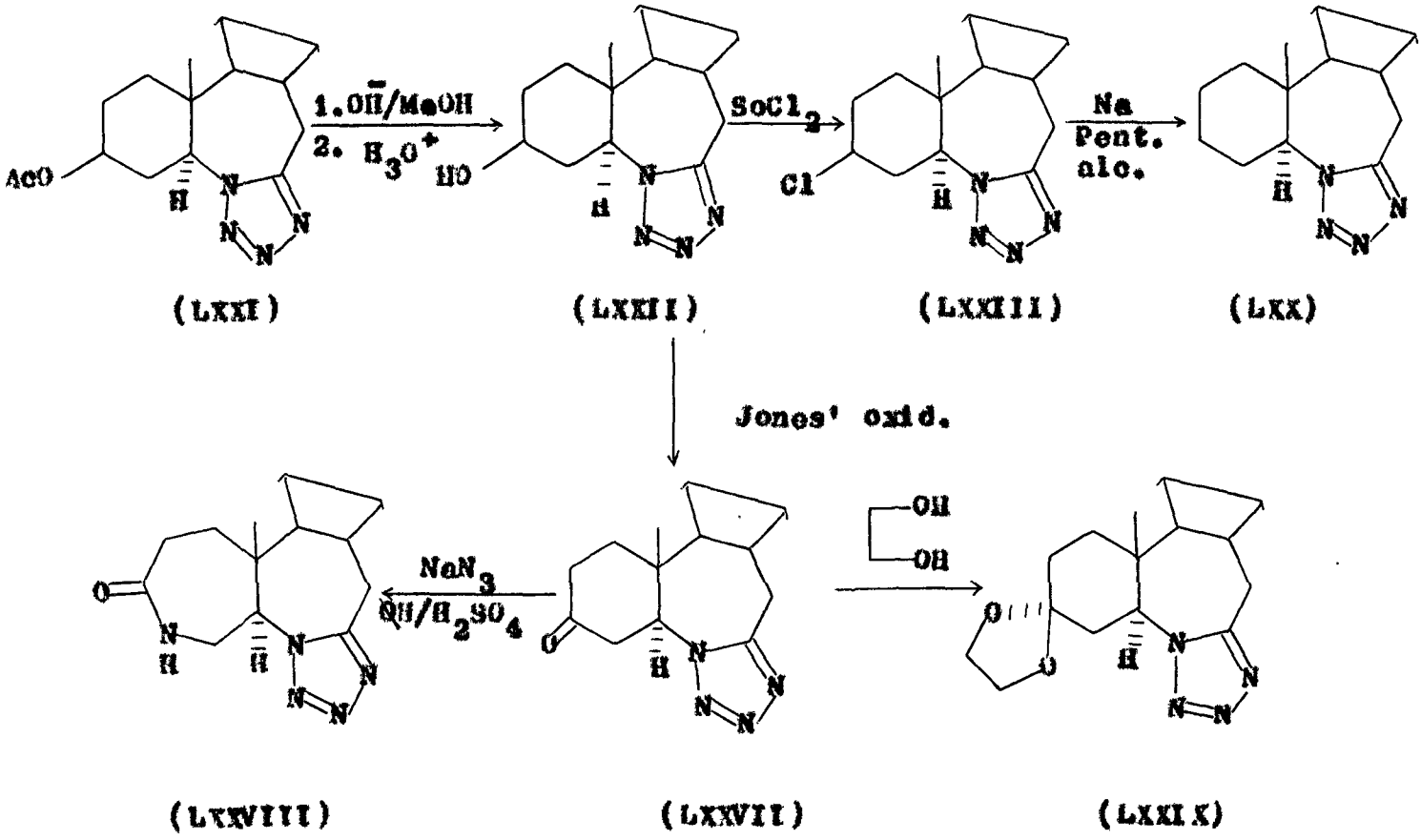


(LXX) R, H
(LXXI) R, OAc
(LXXII) R, OH
(LXXIII) R, Cl



(LXXIV) R, H
(LXXV) R, OH
(LXXVI) R, Cl

The structures were characterized by physical methods and chemical conversions as shown below.



(LXXI) (LXXII) (LXXIII) (LXX)

(LXXVIII) (LXXVII) (LXXIX)

The n.m.r. spectra of tetrazoles exhibited some noteworthy features from characterization viewpoint. There was an unusual diamagnetic shift of C13-methyl protons. Moreover, the C7a protons did not appear together as was expected of them. Attempts have been made to rationalize these facts.

PART - III

Mass Spectrometry of steroidal ϵ-lactones

A communication from this laboratory had described the mass spectra of several 6-oxa lactones (XXIII), (XXVII), (XXIX) and (XXXIII) in the cholestane series, the diagnostic feature of which was an intense peak at m/e 318. Even at that time, the limited value of this study was sounded as this peak could also result from the isomeric 7-oxa lactones. The 7-oxa lactones (XIX), (XXVI) and (XXVIII) prepared in the present study (Part-I) were subjected to mass spectral fragmentation and comparison made with the mass spectra of 6-oxa lactones. In contrast to latter, the former showed characteristic M-CH_2O peaks. This radical difference in the mass spectral behaviour of 6- and 7-oxa lactones can be made use of in distinguishing between these classes of steroidal compounds.

The following table summarises the relevant points of difference between 6- and 7-oxa lactones.

Table

	XXIII	XXVI
(1)	M-CO	M-CH_2O; no M-CO peak
(2)	m/e 318 (base peak)	m/e 318 almost negligible
(3)	m/e 262	m/e 262, 261 (almost of same intensity), 260
(4)	- - -	M-68 (loss of C3,C4,C5,C6).

These differences may be considered of diagnostic value since other isomeric lactones (XXVII and XXVIII) and (XVII and XIX) also exhibited analogous behaviour.

THEORETICAL

Oxasteroids

The oxasteroids, which contain oxygen atom inserted in the steroidal ring structure, with therapeutic properties stimulated extensive research and this resulted in the preparation of a variety of oxygen heterocyclic compounds with useful biological activity. These oxasteroids were found to be of great importance as synthetic intermediates in many reactions. As intermediates, they became important for the insertion of labelled oxygen into the steroid nucleus, ring contraction and preparation of methyl derivatives, etc. Several analogues of biologically active steroids were found to retain their activity, atleast to a certain extent. Oxasteroids are usually prepared in the form of an ether, lactone, anhydride and as derivatives of lactones.

Of the various methods in use for the insertion of oxygen atom in carbon framework, the most widely used is perhaps the Baeyer-Villiger oxidation of ketones. This chapter will be mainly concerned with the review of literature on the preparation of oxasteroids by Baeyer-Villiger oxidation of steroidal ketones.

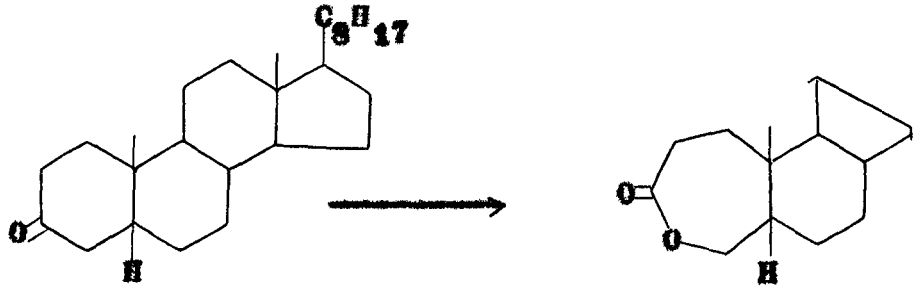
In 1899, Baeyer and Villiger[1] reported the first examples of the peracid oxidation of ketones to corresponding esters or lactones. They used Caro's acid, but in subsequent years several

other peroxyacids also came into use, of which the principal ones are peracetic, trifluoroperacetic, perbenzoic and monoperphthalic acids. Reaction can also be brought about by hydrogen peroxide in weakly basic solution. Since that time this type of oxidation has found a wide variety of important synthetic and degradative applications. An excellent review on the subject is given by Hassall[2]. Thus peracids have been used to synthesise a variety of steroid and terpene lactones as well as lactones involving medium and large rings which are virtually unobtainable by other means.

Baeyer-Villiger oxidation of steroidal ketones

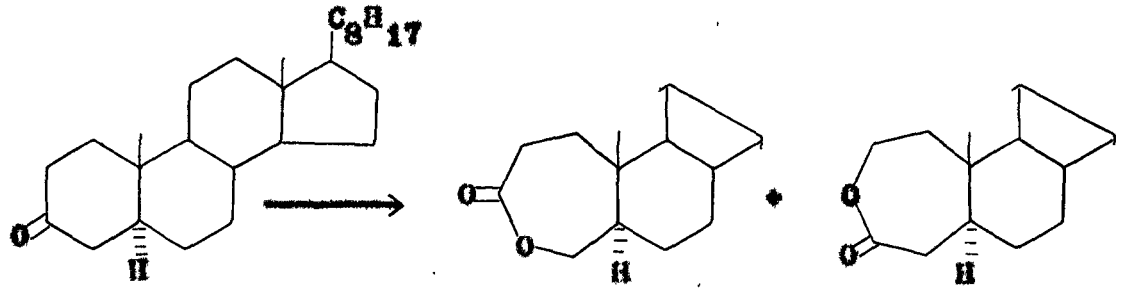
A. Saturated Ketones

In 1913, Gardner and Godden[3] reported that 5β-cholestan-3-one(I) on heating with ammonium persulphate and acetic acid furnished a single product, 4-oxa-A-homo-5β-cholestan-3-one(II).



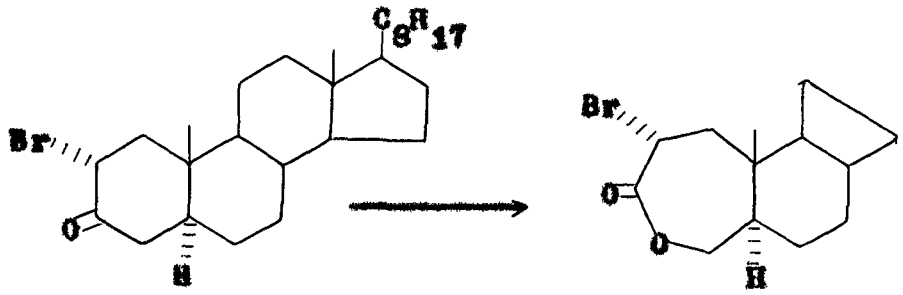
(I) (II)

Ellis and Gardner[4] have observed that 5α-cholestan-3-one(III) on heating with ammonium persulphate and aqueous acetic acid afforded 4-oxa-A-homo-5α-cholestan-3-one(IV) and its isomer, 3-oxa-A-homo-5α-cholestan-4-one(V).



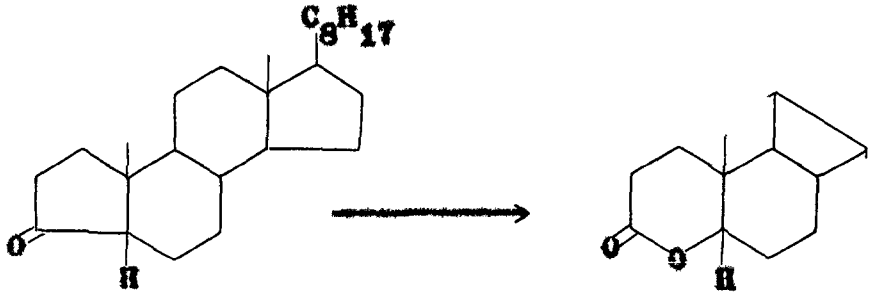
(III) (IV) (V)

Bolliger and Courtney[5] have shown that 2α-bromo-5α-cholestan-3-one(VI), under Baeyer-Villiger conditions, gave a single lactone, 2α-bromo-4-oxa-A-homo-5α-cholestan-3-one(VII).



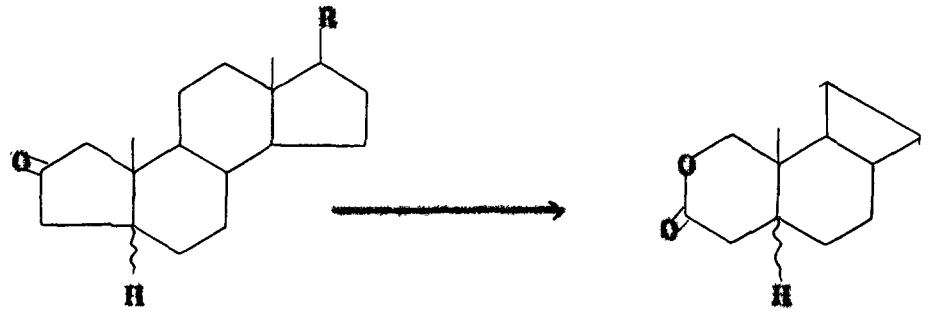
(VI) (VII)

Edward and Morand[6] subjected A-nor-5β-cholestan-3-one (VIII) to peracetic acid oxidation and reported to have obtained a single lactone, 4-oxa-5β-cholestan-3-one (IX).



(VIII) (IX)

Hara[7] has shown that the Baeyer-Villiger oxidation of some 3-keto-A-norcompounds in the cholestane (X, XI) and androstane (XII, XIII) series gave exclusively the corresponding 2-oxasteroids (XIV, XV) and (XVI, XVII). Interestingly, no 3-oxa-isomers were obtained.

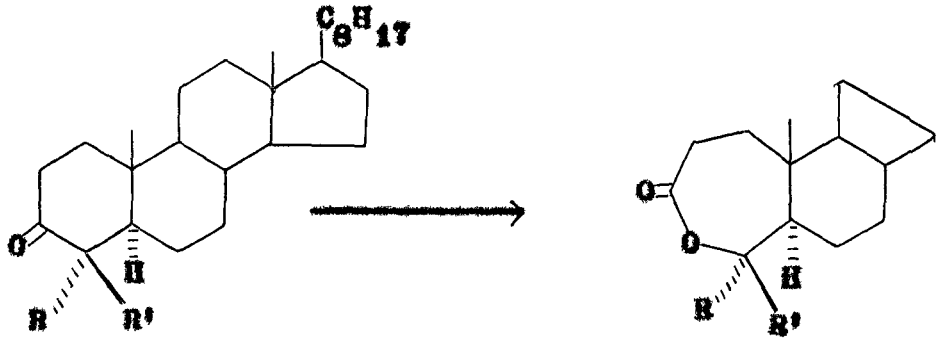


(X) 5α-H; R, C_8H_{17}
(XI) 5β-H; R, C_8H_{17}
(XII) 5α-H; R, H
(XIII) 5β-H; R, H

(XIV) 5α-H; R, C_8H_{17}
(XV) 5β-H; R, C_8H_{17}
(XVI) 5α-H; R, H
(XVII) 5β-H; R, H

Rosenthal et al.[8] have reported that Baeyer-Villiger oxidation of 4α- and 4β-methyl-5α-cholestan-3-ones (XVIII, XIX) and 4,4-dimethyl-5α-cholestan-3-one (XX) with m-chloroperbenzoic acid afforded the ε-lactones, 4aα-methyl-4-oxa-A-homo-

5α-cholestan-3-one (XXI), 4aβ-methyl-4-oxa-A-homo-5α-cholestan-3-one (XXII) and 4a,4a-dimethyl-4-oxa-A-homo-5α-cholestan-3-one (XXIII), respectively, formed by migration of the more highly substituted carbon atom.



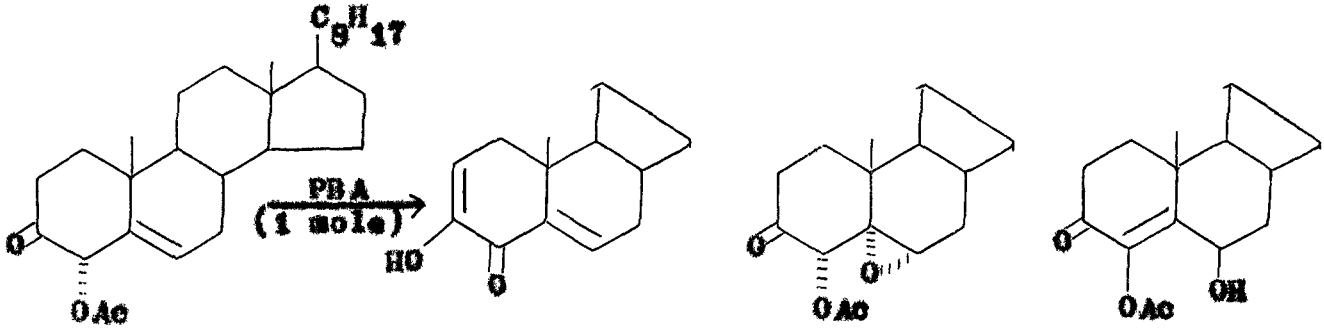
(XVIII) R, CH_3; R',H
(XIX) R,H; R',CH_3
(XX) R,CH_3; R',CH_3

(XXI) R, CH_3; R',H
(XXII) R,H; R',CH_3
(XXIII) R,CH_3; R',CH_3

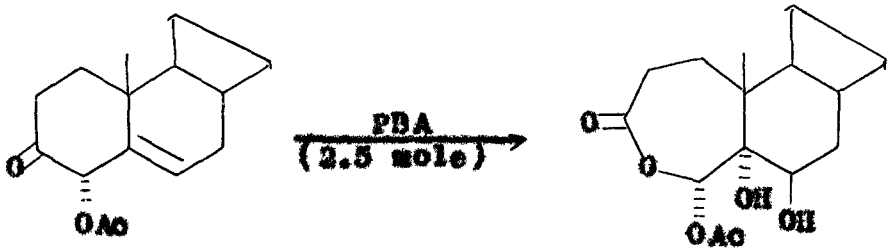
Holker et al.[9] performed the Baeyer-Villiger oxidation of (XX) with m-chloroperbenzoic acid in the presence of a mineral acid as catalyst and interestingly, obtained the unexpected lactone (XXI) with the loss of a C4-methyl group. In the absence of a mineral acid catalyst, they obtained the lactone (XXIII) alone as reported by Rosenthal et al.[8]

Recently, Ahmad et al.[10] have performed the Baeyer-Villiger oxidation of 4α-acetoxycholest-5-en-3-one (XXIV) with perbenzoic acid as oxidant and p-toluenesulphonic acid monohydrate as catalyst. The treatment of (XXIV) with perbenzoic acid (1 mole equivalent) for 56 hours provided 3-hydroxycholesta-2,5-dien-4-one (XXV), 5,6α-epoxy-4α-acetoxy-5α-cholestan-3-one (XXVI) and

6β-hydroxy-4-acetoxycholest-4-en-3-one (XXVII). With an excess of perbenzoic acid (2.5 mole equivalent), (XXIV) furnished a single product, 5,6β-dihydroxy-4a-ϰ-acetoxy-4-oxa-A-homo-5-ϰ-cholestan-3-one (XXVIII).



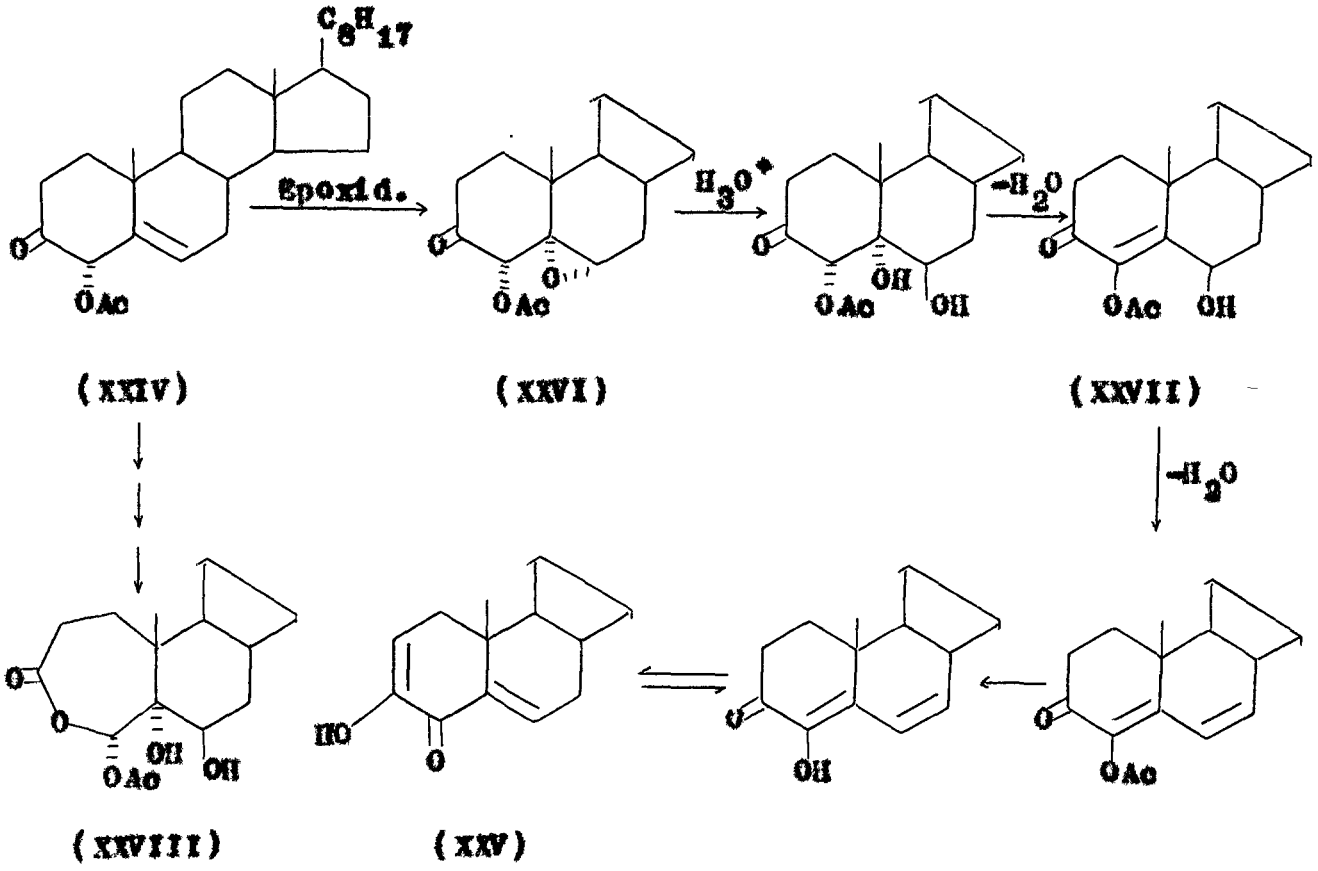
(XXIV) (XXV) (XXVI) (XXVII)



(XXIV) (XXVIII)

The formation of products (XXV-XXVIII) from (XXIV), under Baeyer-Villiger conditions, has been proposed to occur according to Scheme-1.

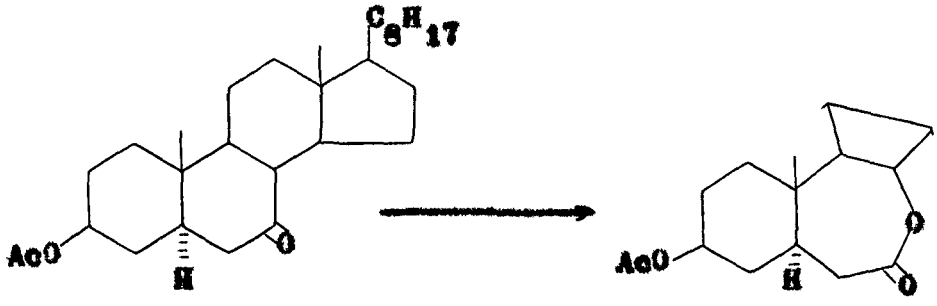
Scheme - 1



(XXIV) (XXVI) (XXVII)

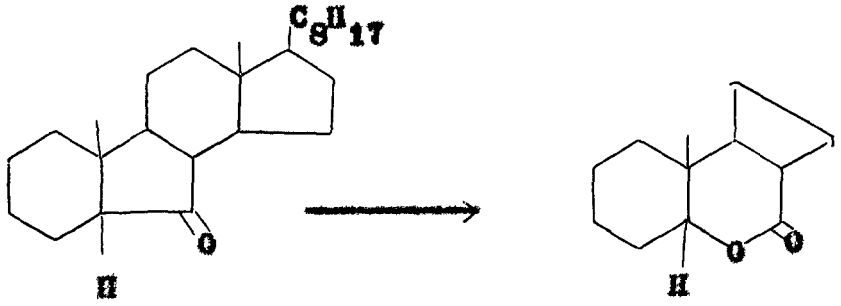
(XXVIII) (XXV)

Plattner et al.[11] have reported that Baeyer-Villiger oxidation of 3β-acetoxy-5α-cholestan-7-one (XXIX) with perbenzoic acid furnishes a single lactone, 3β-acetoxy-7a-oxa-8-homo-5α-cholestan-7-one (XXX).



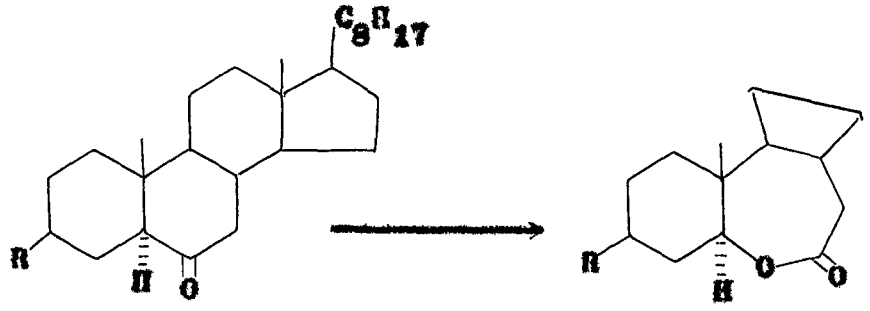
(XXIX) (XXX)

Atwater[12] accomplished the trifluoroperacetic acid oxidation of B-nor-5β-cholestan-7-one (XXXI) and obtained a single lactone, 6-oxa-5β-cholestan-7-one (XXXII).



(XXXI) (XXXII)

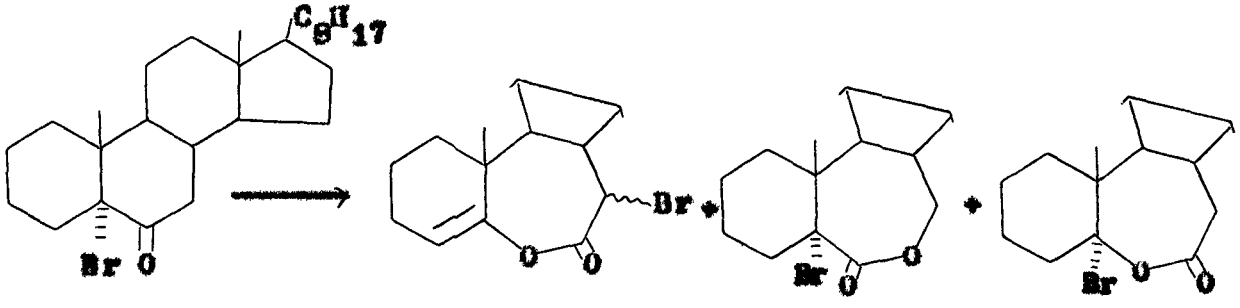
Fonken and Miles[13] performed the Baeyer-Villiger oxidation of 5α-cholestan-6-one (XXXIII) and its 3β-acetoxy derivative (XXXIV) with perbenzoic acid and reported the exclusive formation of the corresponding 6-oxa lactones (XXXV) and (XXXVI), formed by preferential migration of a more substituted C5 relative to C7.



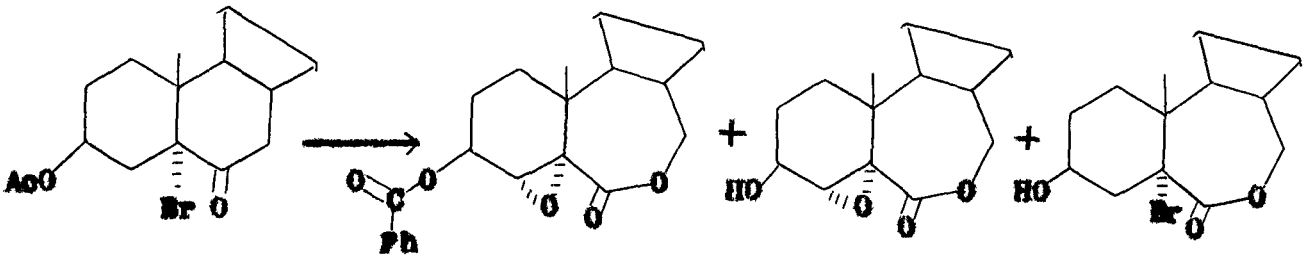
(XXXIII) R, H
(XXXIV) R, OAc

(XXXV) R, H
(XXXVI) R, OAc

In order to investigate the effect of a substituent in the close vicinity of 6-keto moiety on the course of reaction, Ahmad and coworker[14] carried out the Baeyer-Villiger oxidation of 5α-bromocholestan-6-one (XXXVII) and 3β-acetoxy-5α-bromocholestan-6-one (XXXVIII) with perbenzoic acid (p-toluenesulphonic acid monohydrate as catalyst). After a week's reaction time (XXXVII) afforded 7a-bromo-6-oxa-B-homocholest-4-en-7-one (XXXIX), 7-oxa-B-homo-5α-bromocholestan-6-one (XL) and 6-oxa-B-homo-5α-bromocholestan-7-one (XLI), while (XXXVIII) after 24 hours gave 3β-benzoyloxy-4α,5-epoxy-7-oxa-B-homo-5α-cholestan-6-one (XLII), 3β-hydroxy-4α,5-epoxy-7-oxa-B-homo-5α-cholestan-6-one (XLIII) and 3β-hydroxy-3α-bromo-7-oxa-B-homocholestan-6-one (XLIV).



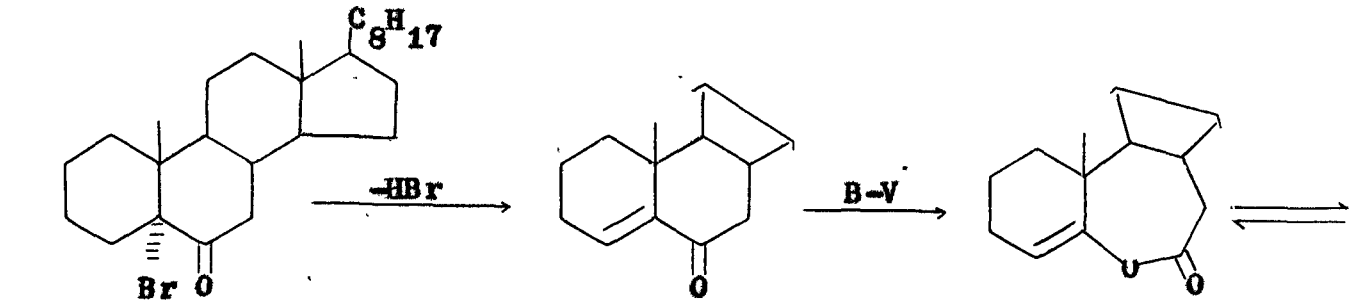
(XXXVII) (XXXIX) (XL) (XLI)



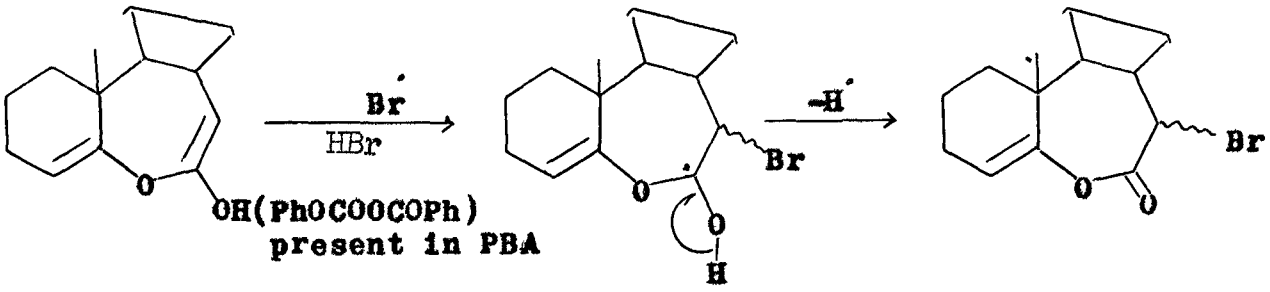
(XXXVIII) (XLII) (XLIII) (XLIV)

A tentative mechanism, involving the attack of bromine radical at one stage, was proposed for the conversion of (XXXVII) ⟶ (XXXIX) (Scheme-2).

Scheme - 2

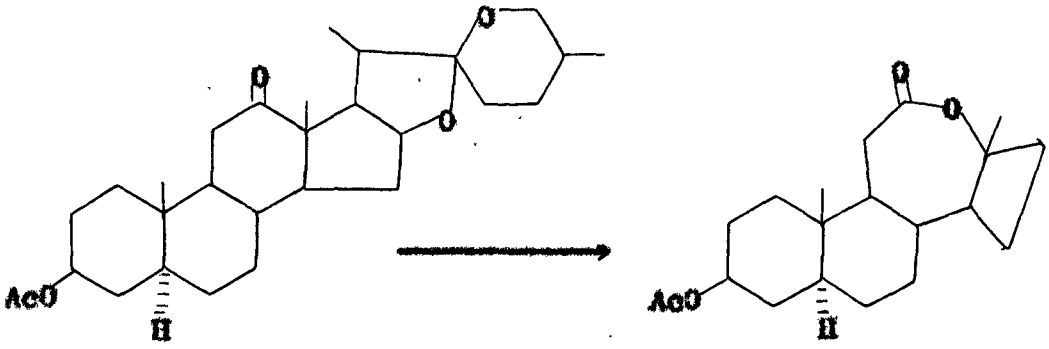


(XXXVII)



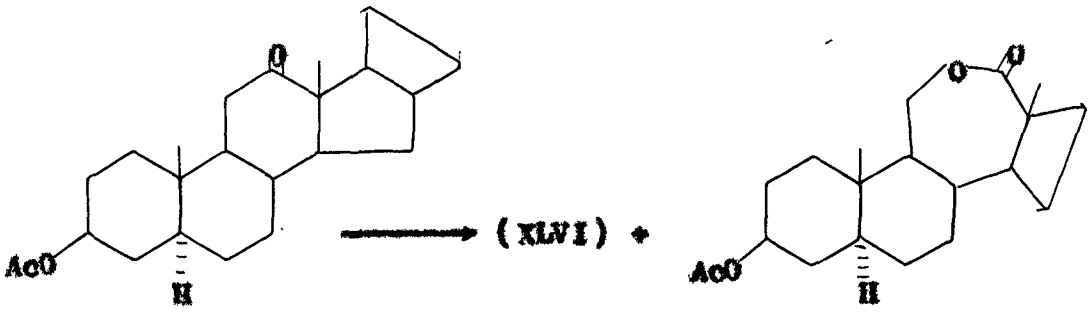
(XXXIX)

Rothman et al.[15] have reported the formation of a single lactone (XLVI) from the Baeyer-Villiger oxidation of hecogenin acetate (XLV) using peracetic and perbenzoic acids.



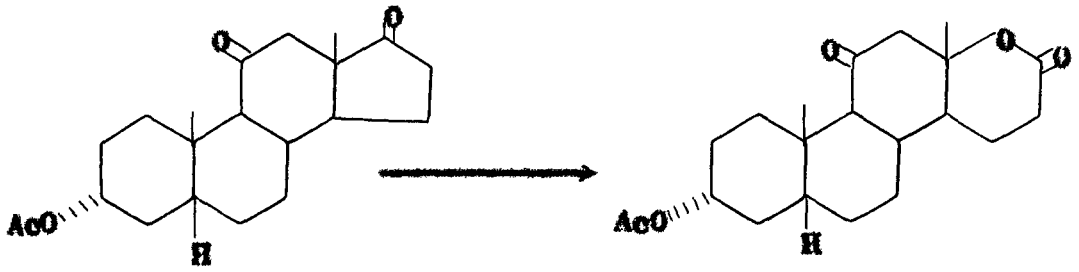
(XLV) (XLVI)

Bladon and McMeekin[16] reinvestigated the Baeyer-Villiger oxidation of hecogenin acetate (XLV) with peracetic acid and reported the formation of isomeric lactone (XLVII) also along with (XLVI).



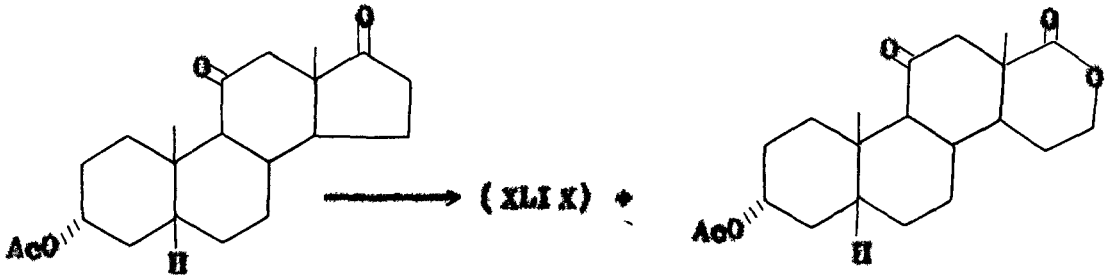
(XLV) (XLVI) + (XLVII)

Wendler et al.[17] have shown that perbenzoic acid oxidation of 3α-acetoxy-5β-androstane-11,17-dione (XLVIII) afforded the corresponding 17-oxa lactone (XLIX) only.



(XLVIII) (XLIX)

Later, Lardon et al.[18] repeated the perbenzoic acid oxidation of (XLVIII) and observed the formation of the isomeric lactone (L) along with (XLIX).



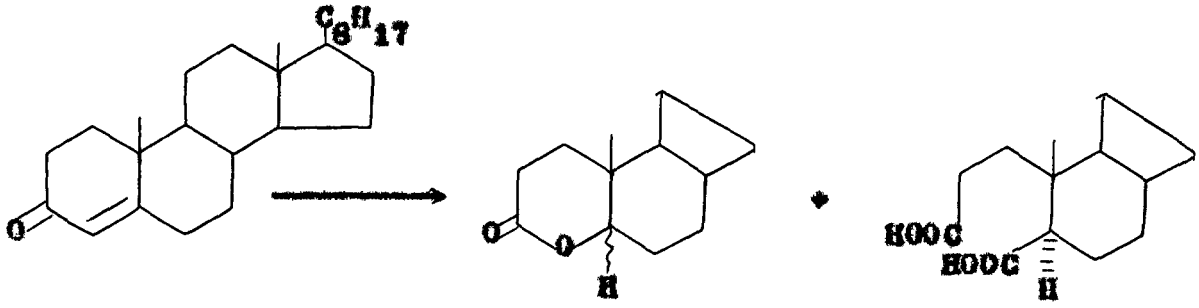
(XLVIII) (L)

B. <u>α,β-Unsaturated Ketones</u>

Structural elements other than carbonyl group may be attacked under the conditions used for the Baeyer-Villiger reaction. The susceptibility of olefin linkages to oxidation by peracids is well-known. Peracid oxidation of cyclic α,β-unsaturated ketones may lead to enol lactones, epoxy lactones and epoxy ketones or products derived from them as a consequence of extended side reactions[19-23]. However, the peracid oxidations of Δ^4-3-ketosteroids have been shown to provide a wide array

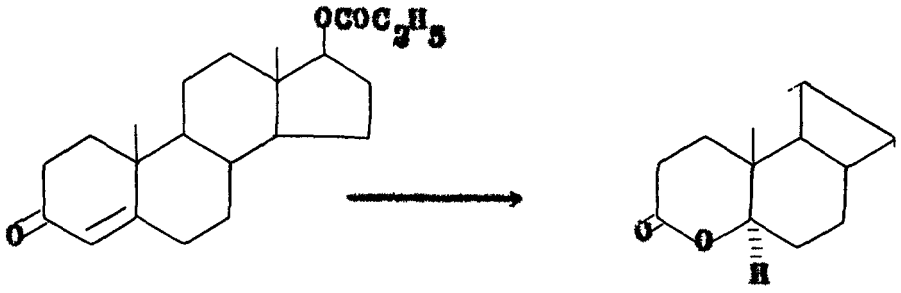
of products depending upon the reaction conditions and the peracid used.

In 1941, Salamon[24] observed that oxidation of cholest-4-en-3-one (LI) with potassium persulphate and sulphuric acid yielded a neutral compound (LII) and an unidentified acid. In 1950, Turner[25] repeated Salamon's experiments and identified the neutral product as 4-oxa-5α-cholestan-3-one (LII-a) and the acid as dihydro Diels' acid (LIII).

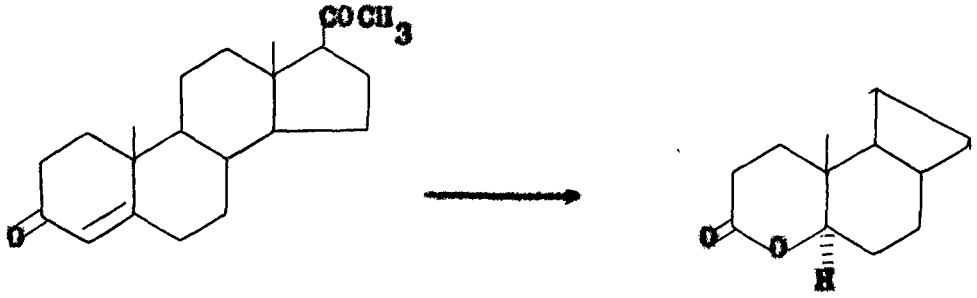


(LI) (LII) (LII-a) 5α-H (LIII)

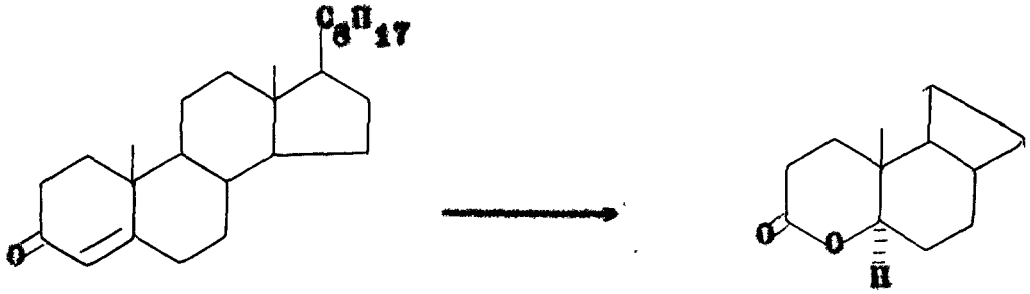
Petit and Kasturi[26] have performed the peroxy sulphuric acid oxidation of testosterone propionate (LIV), progesterone (LV) and cholest-4-en-3-one (LI) and obtained the 4-oxa-3-keto-5α-steroids (LVI), (LVII) and (LII-a), respectively.



(LIV) (LVI)

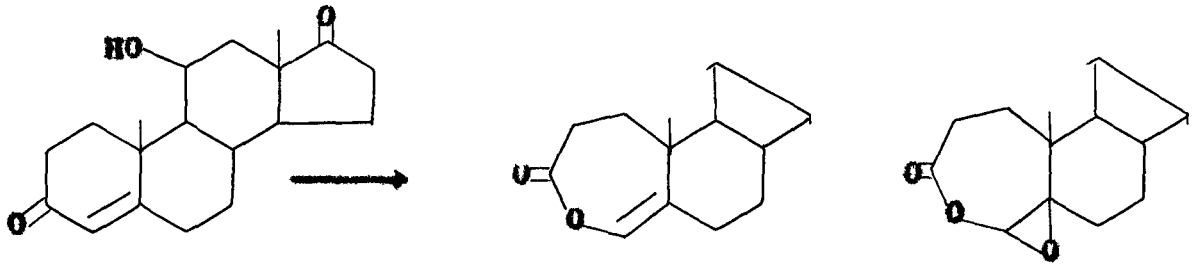


(LV) (LVII)



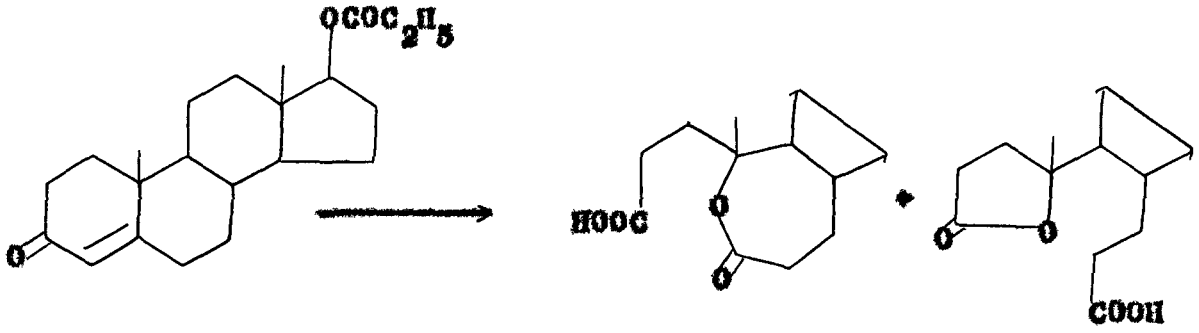
(LI) (LII-a)

Caspi et al.[37] carried out the Baeyer-Villiger oxidation of 11β-hydroxyandrost-4-ene-3,17-dione (LVIII) which afforded the enol lactone, 11β-hydroxy-4-oxa-A-homoandrost-4a-ene-3, 17-dione (LIX) and a small quantity of the epoxy lactone, 11β-hydroxy-4-oxa-4aβ,5-epoxy-A-homo-5β-androstane-3,17-dione (LX).



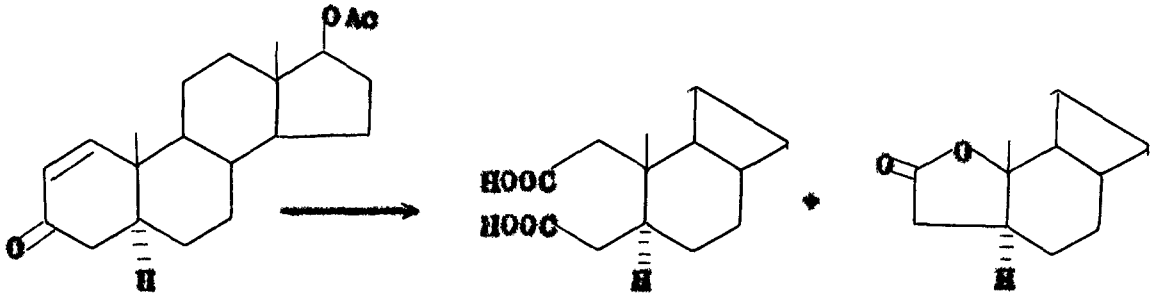
(LVIII) (LIX) (LX)

Caspi and Balasubramanyan[23] treated testosterone propionate (LIV) with hydrogen peroxide in the presence of selenium dioxide in t-butyl alcohol which provided the ∈-lactone carboxylic acid (LXI) and the γ-lactone acid (LXII).



(LIV) (LXI) (LXII)

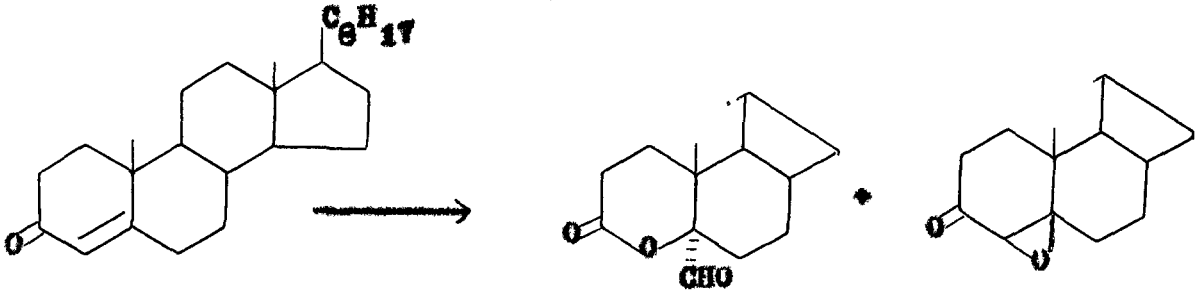
Caspi and Shimizu[29] also performed the Baeyer-Villiger oxidation of 17β-acetoxy-5α-androst-1-en-3-one (LXIII) with hydrogen peroxide in the presence of selenium dioxide which provided 17β-acetoxy-3,3-seco-5α-androstan-1,4-dicarboxylic acid (LXIV) and the γ-lactone (LXV).



(LXIII) (LXIV) (LXV)

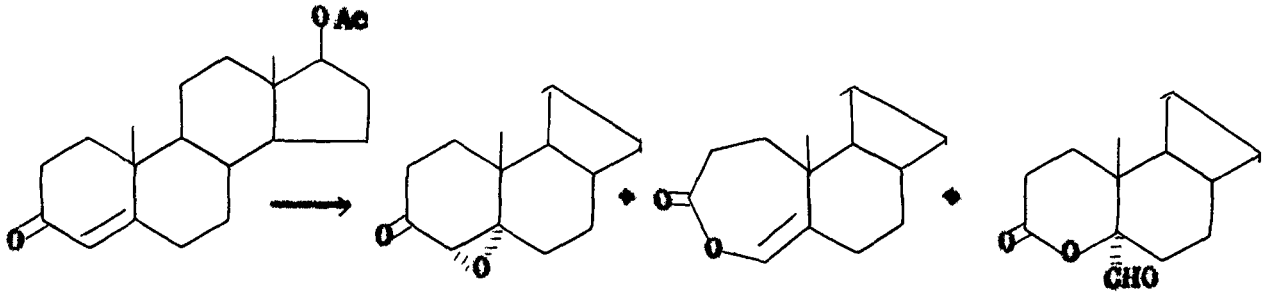
Pinhey and Schaffner[30] accomplished the Baeyer-Villiger oxidation of cholest-4-en-3-one (LI) with trifluoroperacetic acid

in buffer solution and obtained 5-formyl-4-oxa-5α-cholestan-3-one (LXVI) and 4β,5-epoxy-5β-cholestan-3-one (LXVII).



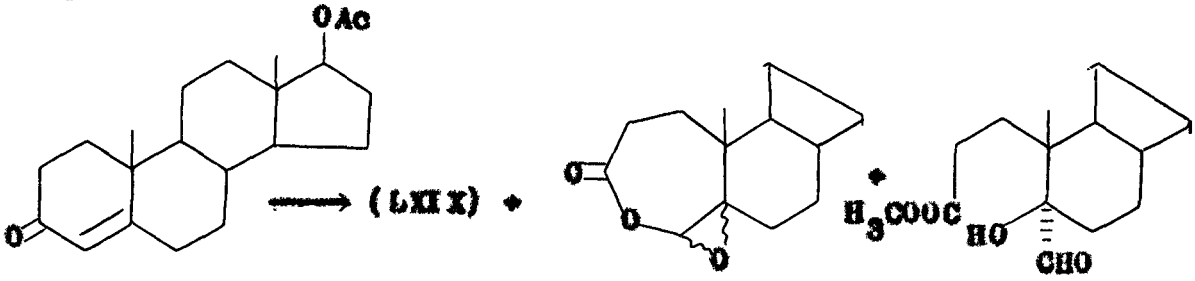
(LI) (LXVI) (LXVII)

Mazur et al.[31] carried out a detailed study of the oxidation of testosterone acetate (LXVIII) with perbenzoic acid and m-chloroperbenzoic acid in the presence of perchloric acid as catalyst. They observed that the products obtained and their ratio depended on the quantity of peracid used, catalyst and on the reaction time. Oxidation of (LXVIII) with perbenzoic acid (1 mole equivalent) in the presence of anhydrous perchloric acid for 12 hours gave 17β-acetoxy-4α,5α-epoxyandrostan-3-one (LXIX), 17β-acetoxy-4-oxa-A-homoandrost-4a-en-3-one (LXX) and 17β-acetoxy-5-formyl-4-oxa-5α-androstan-3-one (LXXI).



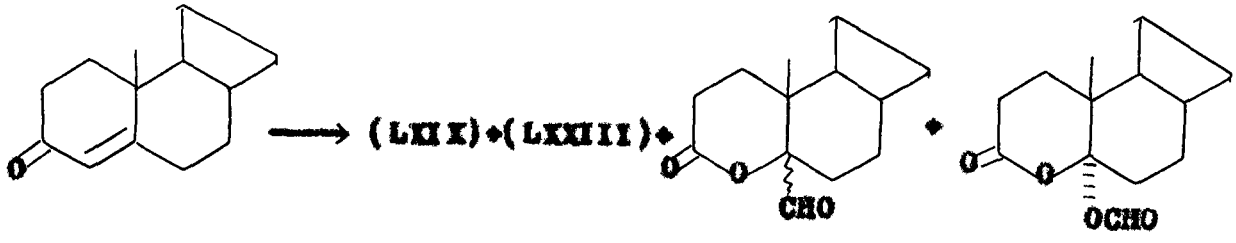
(LXVIII) (LXIX) (LXX) (LXXI)

When (LXVIII) was treated with perbenzoic acid (2 mole equivalent) in the presence of anhydrous perchloric acid as catalyst (84 hours), the products obtained were the epoxy ketone (LXIX), 17β-acetoxy-4a,5-epoxy-4-oxa-A-homoandrostan-3-ones (LXXII, LXXIII) and methyl 17β-acetoxy-3,3-seco-4-nor-5β-hydroxy-5α-formylandrostan-3-oate (LXXIV).



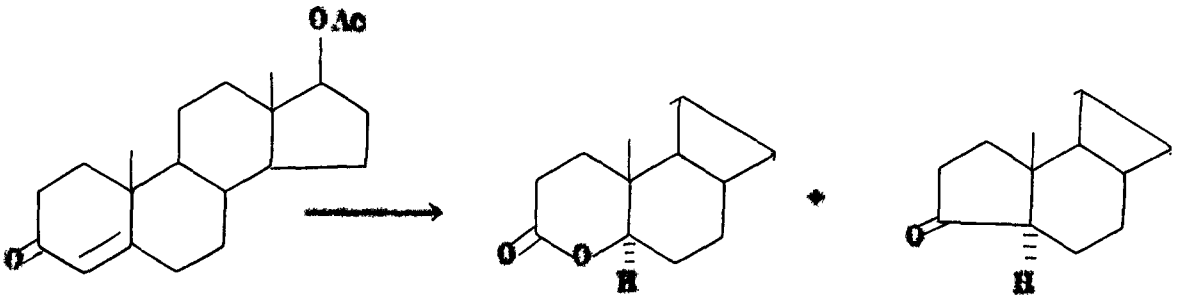
(LXVIII) (LXXII) 4a ,5 (LXXIV)
(LXXIII) 4a ,5

The oxidation of (LXVIII) with an excess of perbenzoic acid (4 mole equivalent) in the presence of anhydrous perchloric acid as catalyst (84 hours), gave the epoxy ketone (LXIX), the epoxy lactone (LXXIII), 5α- and 5β-formyl-δ-lactones (LXXI, LXXV), and 17β-acetoxy-5-formate-4-oxa-5α-androstan-3-one(LXXVI).



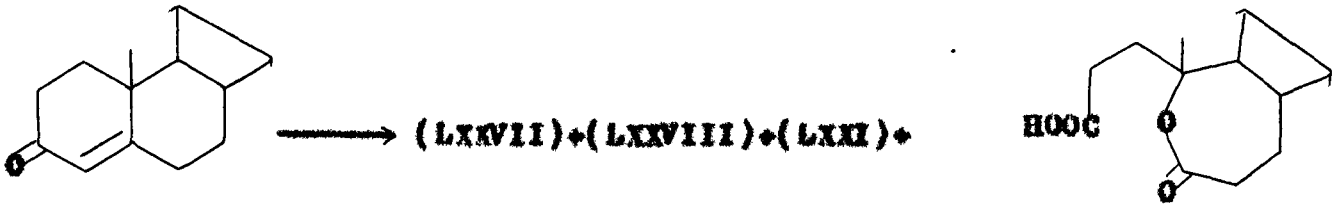
(LXVIII) (LXXI) 5α- (LXXVI)
(LXXV) 5β-

When the reaction with perbenzoic acid (2 mole equivalent) was carried out in the presence of aqueous perchloric acid (12 hours), the δ-lactone (LXXVII) and 17β-acetoxy-A-norandrostan-3-one (LXXVIII) were obtained.



(LXVIII) (LXXVII) (LXXVIII)

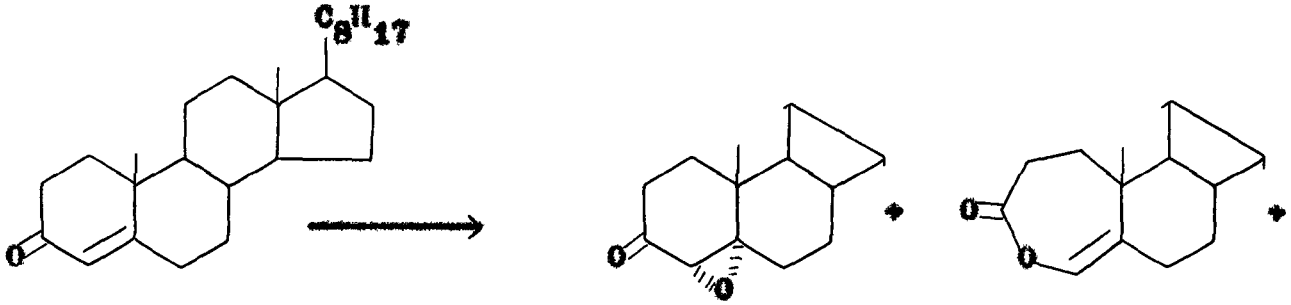
When m-chloroperbenzoic acid was used as the oxidising agent and aqueous perchloric acid as the catalyst (12 hours), (LXVIII) afforded the δ-lactone (LXXVII), the A-norketone (LXXVIII), the lactone aldehyde (LXXI) and 17β-acetoxy-3,5-seco-4-nor-5-oxa-3-homoandrostan-6-one-2-carboxylic acid (LXXIX).



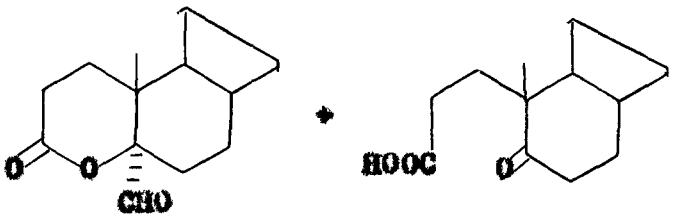
(LXVIII) (LXXIX)

Pinhey and Schaffner[32], in continuation of their previous study[30], oxidised (LI) with perbenzoic acid in the presence of

catalytic amounts of anhydrous perchloric acid and reported the isolation of 4α,5-epoxy-5α-cholestan-3-one (LXXX), 4-oxa-A-homocholest-4a-en-3-one (LXXXI), 5-formyl-4-oxa-5α-cholestan-3-one (LXVI) and 3,5-seco-4-norcholestan-5-one-3-carboxylic acid (LXXXII).

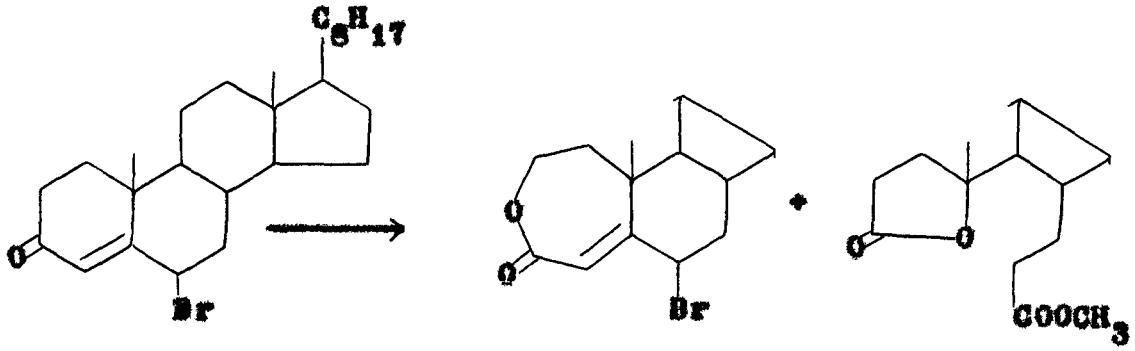


(LI) (LXXX) (LXXXI)



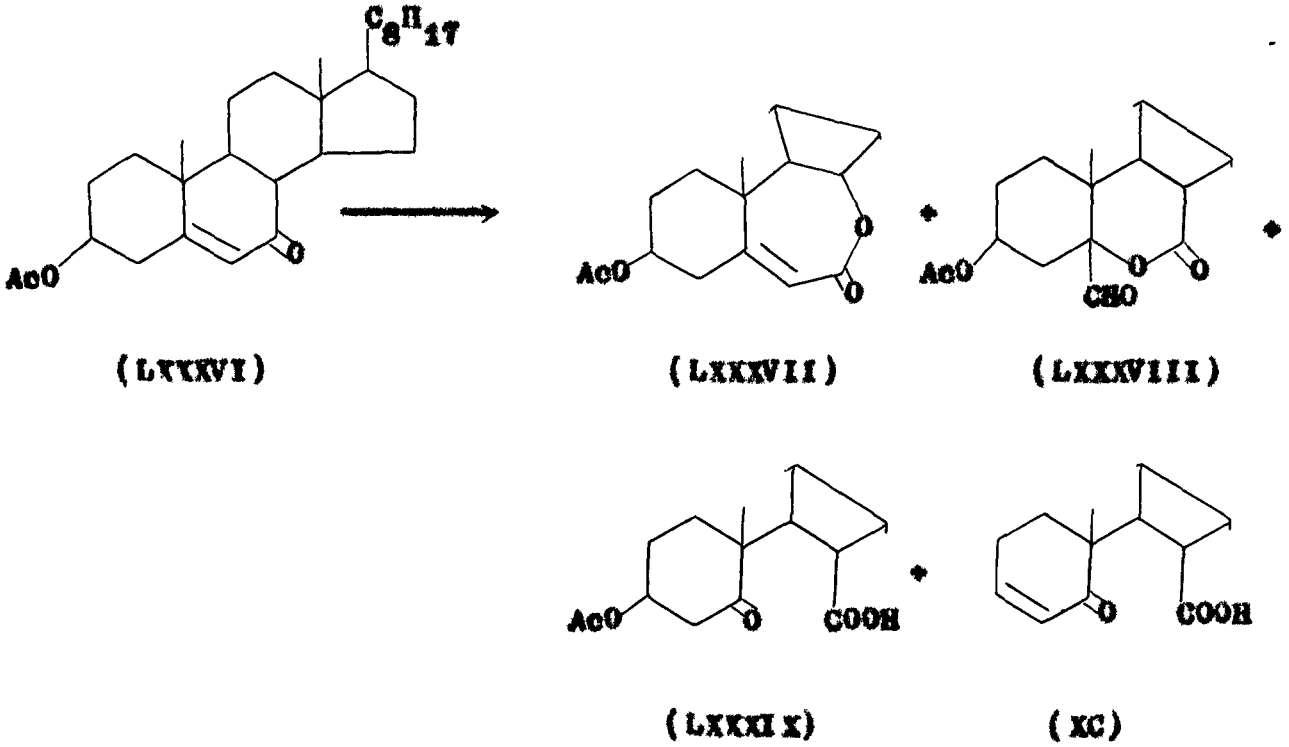
(LXVI) (LXXXII)

Ahmad et al.[33] subjected 6β-bromocholest-4-en-3-one(LXXXIII) to perbenzoic acid oxidation using p-toluenesulphonic acid monohydrate as catalyst. The choice of the substrate (LXXXIII) was governed by the desire to know the effect of a substituent in close vicinity of 4-en-3-one moiety on the course of reaction and product distribution. The products obtained were 6β-bromo-3-oxa-A-homocholest-4a-en-4-one (LXXXIV), a product of primary oxidation and the γ-lactone methyl ester (LXXXV), a product of extended reaction.



(LXXXIII) (LXXXIV) (LXXXV)

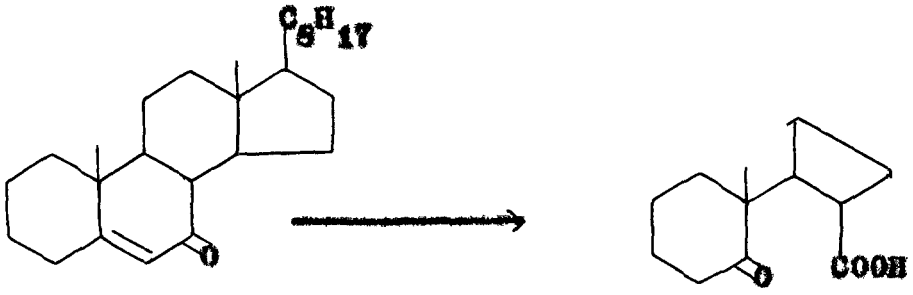
In view of the absence of any analogous study of the peracid oxidation of 5-en-7-ones relative to 4-en-3-ones, Ahmad et al.[34] attempted the Baeyer-Villiger oxidation of 3β-acetoxycholest-5-en-7-one (LXXXVI), using perbenzoic acid as oxidant and p-toluenesulphonic acid monohydrate as catalyst. This reaction afforded 3β-acetoxy-7a-oxa-8-homocholest-5-en-7-one (LXXXVII), 3β-acetoxy-6-oxa-5-formyl-5β-cholestan-7-one (LXXXVIII) and a mixture of the secoacids, 3β-acetoxy-5-keto-5,7-seco-6-norcholestan-7-oic acid (LXXXIX) and 5-keto-5,7-seco-6-norcholest-3-en-7-oic acid (XC).



(LXXXVI) (LXXXVII) (LXXXVIII)

(LXXXIX) (XC)

Oxidation of cholest-5-en-7-one (XCI) under similar conditions furnished a single compound, 5-keto-5,7-seco-6-norcholestan-7-oic acid (XCII)[34].



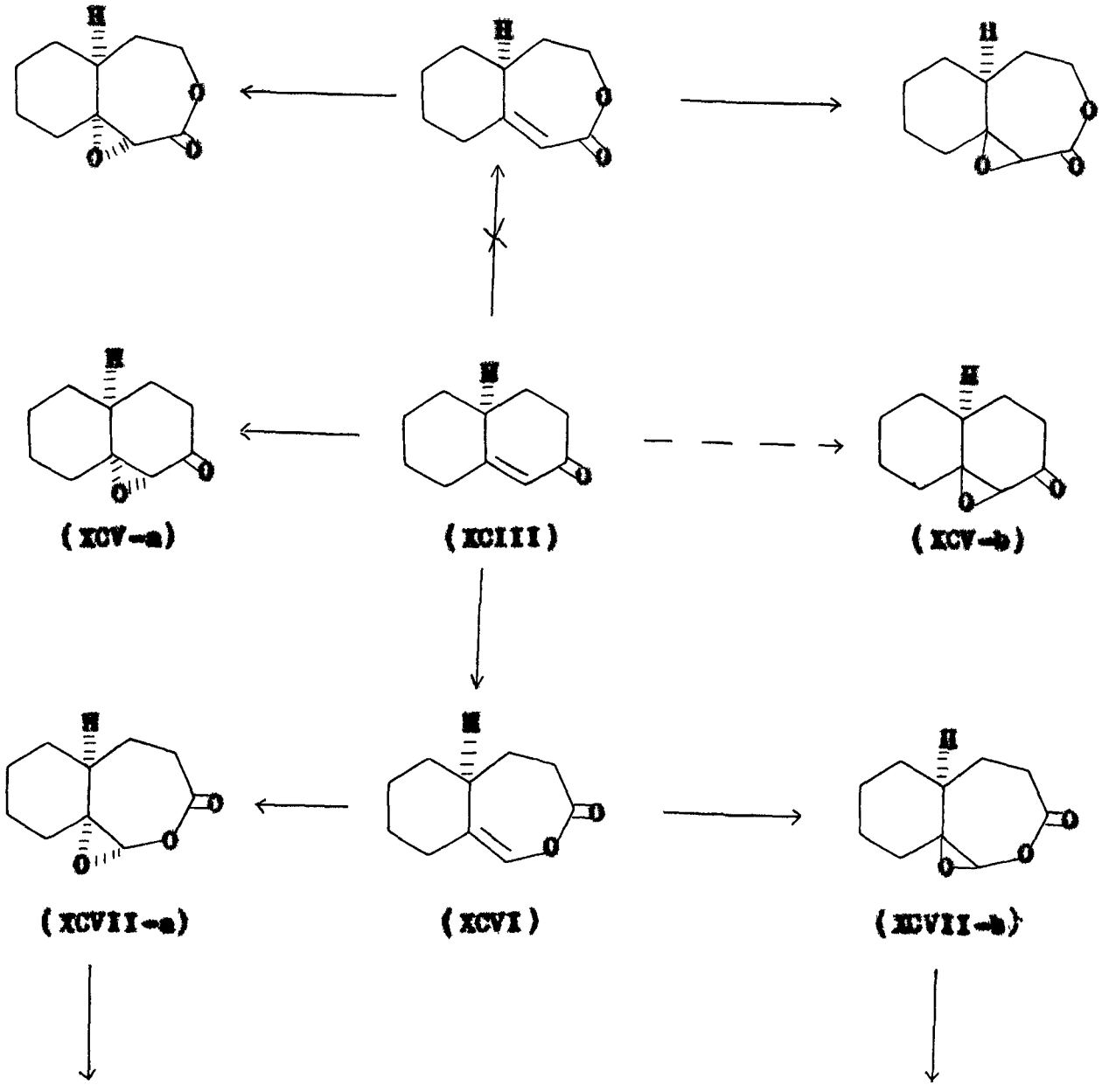
(XCI) (XCII)

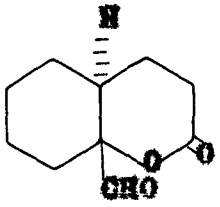
As it is evident, several Δ^4-3-ketosteroids have been subjected to Baeyer-Villiger oxidation reactions. These reactions provided a variety of products and distribution of these products has been studied with variance of temperature, reaction times, catalyst and equivalents of oxidizing agents used. These oxidations helped in isolation of many intermediates, particularly the oxidation of testosterone acetate (LXVIII) with perbenzoic acid, in which all of the intermediates were isolated and characterized.

In order to isolate similar intermediates from simple α,β-unsaturated cyclic ketones, DeBoer and Ellwanger[35] recently carried out the Baeyer-Villiger oxidation of $\Delta^{1(9)}$-octalone-2-(XCIII) and $\Delta^{1(8)}$-indanone-2(XCIV) with peracids. They have also attempted to correlate product distribution with the reaction conditions employed.

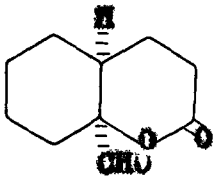
The peracid oxidation of (XCIII) gave the epoxy ketone (XCV-a), the enol lactone (XCVI), the epoxy lactones (XCVII-a, XCVII-b), the aldehyde lactones (XCVIII-a, XCVIII-b) and the formate lactones (XCIX-a, XCIX-b) (Scheme - 3). The relationship indicated by the dashed arrow is only suppositional while those indicated by solid arrows have been observed.

Scheme - 3

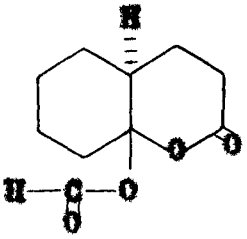




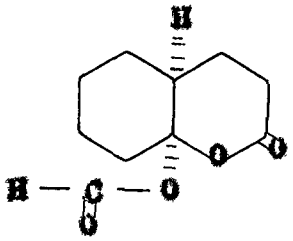
(XCVIII-a)



(XCVIII-b)



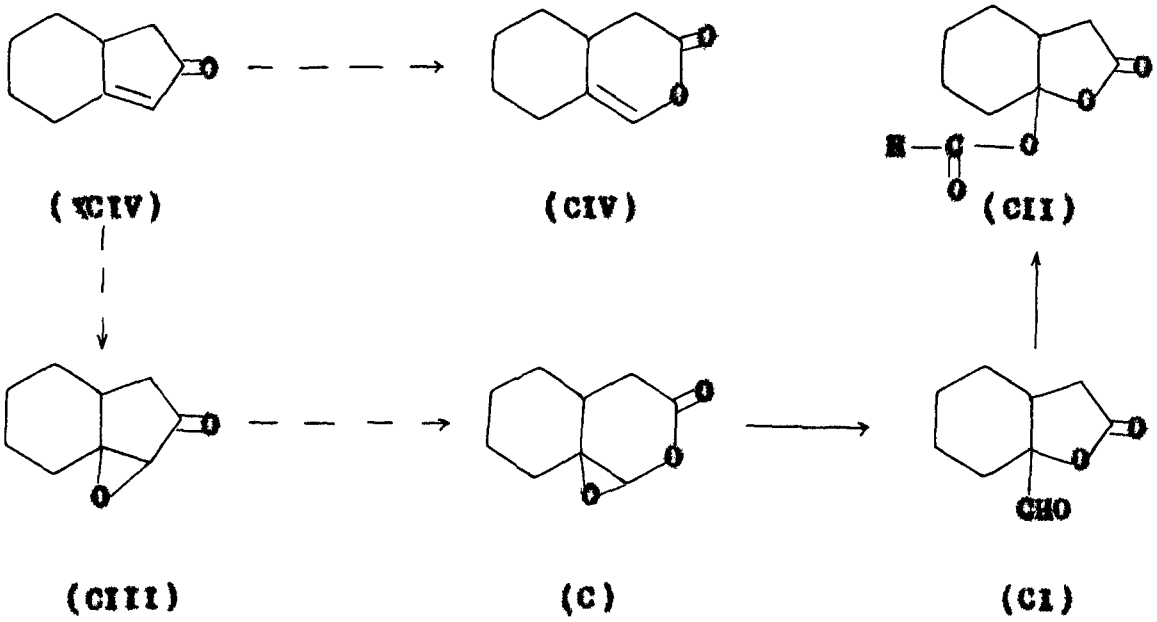
(XCIX-a)



(XCIX-b)

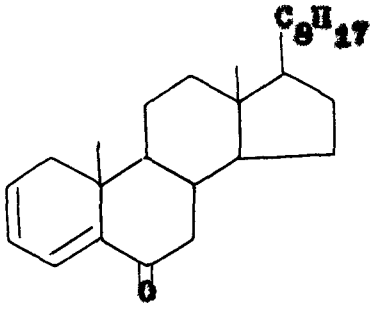
The oxidation of (XCIV) with peracids led to the epoxy lactone (C), the aldehyde lactone (CI) and the formate lactone (CII). The primary oxidation products, the epoxy ketone (CIII) and the enol lactone (CIV), were not detected (Scheme-4).

Scheme - 4

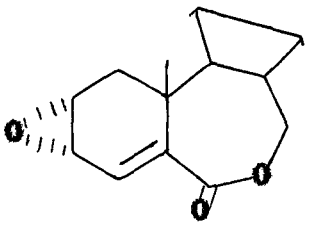


C. Dienone and Rings B and A Seco-5-ketocompounds

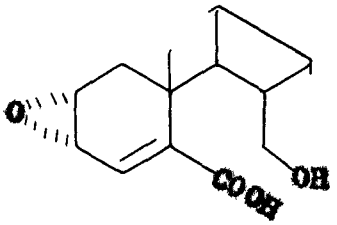
Ahmad and Pillai[36] performed the Baeyer-Villiger oxidation of cholesta-2,4-dien-6-one (CV) with perbenzoic acid (2.5 mole) equivalent) and p-toluenesulphonic acid monohydrate as catalyst which afforded 2α,3α-oxido-5,6-secocholest-4-en-7-ol-6-oic acid (CVII) as the major product of the reaction. Apparently the reaction involved the formation of the intermediate (CVI) which suffered hydrolysis to give (CVII).



(CV)



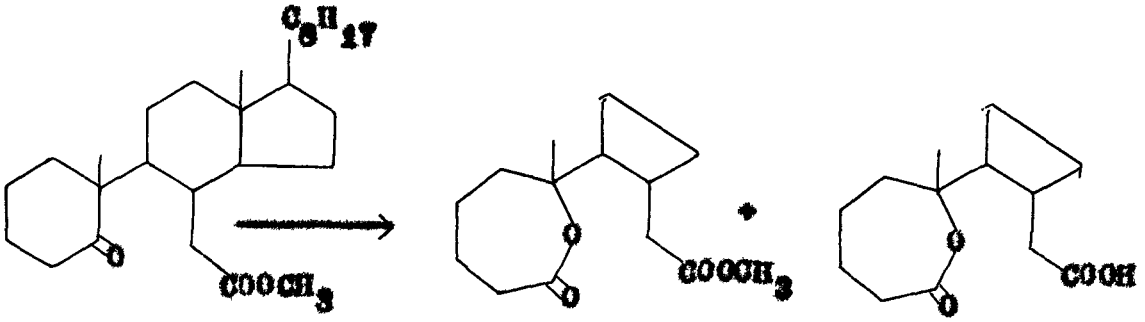
(CVI)



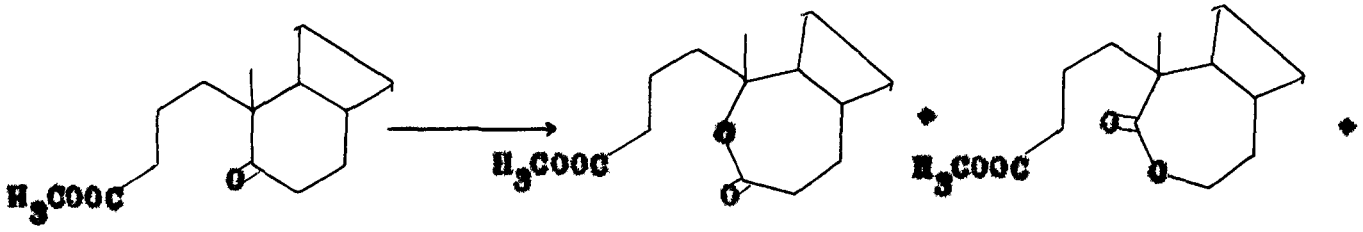
(CVII)

Ahmad and coworkers[37] carried out the perbenzoic acid oxidation of methyl 5-keto-5,6-secocholestan-6-oate (CVIII), methyl 5-keto-4,5-secocholestan-4-oate (CIX) and methyl 5-keto-5,6-secocholest-3-en-6-oate (CX) using p-toluenesulphonic acid monohydrate as catalyst. The compound (CVIII) afforded methyl 5a-oxa-5-keto-5,6-seco-A-homocholestan-6-oate (CXI) and its partial hydrolysis product 5a-oxa-3-keto-5,6-seco-A-homocholestan-6-oic acid (CXII). The compound (CIX) gave isomeric lactones,

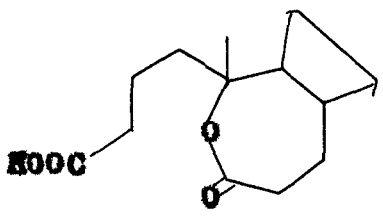
methyl-5-oxa-6-keto-4,5-seco-8-homocholestan-4-oate (CXIII), methyl 6-oxa-5-keto-4,5-seco-8-homocholestan-4-oate (CXIV) and 5-oxa-6-keto-4,5-seco-8-homocholestan-4-oic acid (CXV)(a product of partial hydrolysis of CXIII). Oxidation of (CX) furnished methyl 3α,4α-epoxy-5-keto-5,6-secocholestan-6-oate (CXVI) and methyl 5,6-seco-3α,4α-epoxy-5-keto-5a-oxa-A-homocholestan-6-oate (CXVII).



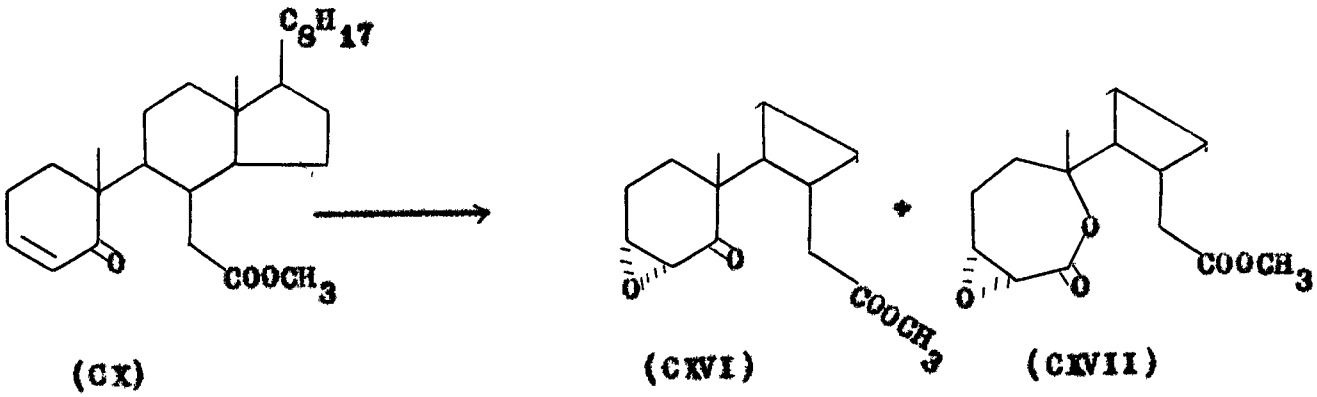
(CVIII) (CXI) (CXII)



(CIX) (CXIII) (CXIV)



(CXV)



(CX) (CXVI) (CXVII)

Table - I

Spectral data of some steroidal lactones

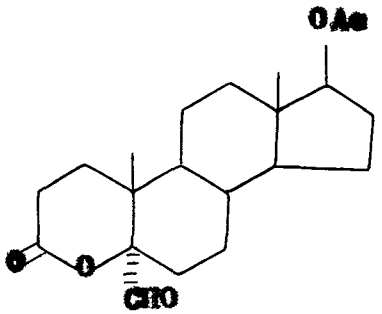
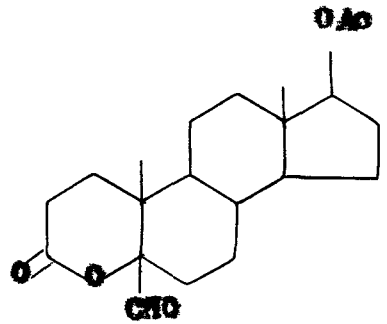
Compound	NMR (δ)	I.R. (cm^{-1})	U.V. nm (log ϵ)	Ref.
 (LXVI)	10.1s(C<u>H</u>O), 2.75m (C2-<u>H</u>$_2$), 1.1s (C10-C<u>H</u>$_3$) and 0.84s (C13-C<u>H</u>$_3$)	1730 (δ-lactone, aldehydic and acetate carbonyls)	-	31
 (LXXV)	9.67 (C<u>H</u>O), 3.55m (C2-<u>H</u>$_2$), 1.16s (C10-C<u>H</u>$_3$) and 0.81s (C13-C<u>H</u>$_3$)	1745 (δ-lactone, aldehydic and acetate carbonyl)	-	**

Table - I(Contd.)

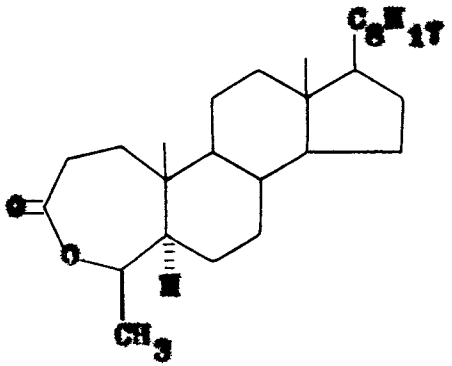
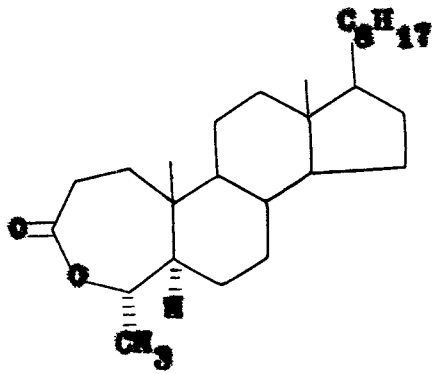
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (XXII)	4.37m (C4a-αH), 1.47d (C4a-$\beta$$CH_3$, J 7 Hz), 1.03s (C10-$CH_3$)	1730 (ϵ-lactone carbonyl)	-	8
 (XXI)	4.5m (C4a-βH), 1.22d (C4a-$\alpha$$CH_3$, J 7 Hz), 0.93s (C10-$CH_3$).	1728 (ϵ-lactone carbonyl)	-	9

Table - I(Contd.)

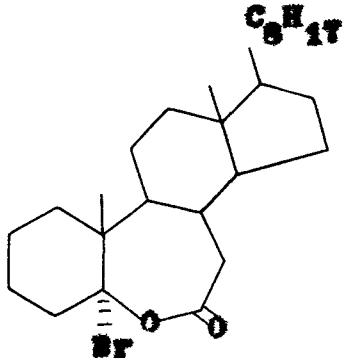
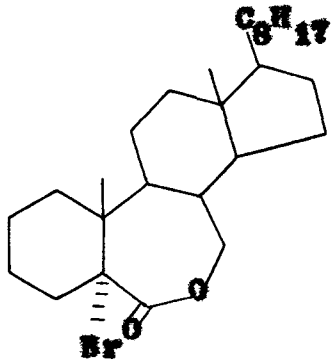
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (XLI)	2.3 mmc (C_7a-$\underline{H}_2$), 0.90s (C_{10}-$C\underline{H}_3$), 0.71s (C_{13}-$C\underline{H}_3$), 0.88 and 0.83 (other methyl protons)	1710 (ϵ-lactone carbonyl)	-	14
 (XL)	4.2 mmc (C_7a-$\underline{H}_2$), 0.90s (C_{10}-$C\underline{H}_3$), 0.70s (C_{13}-$C\underline{H}_3$), 0.86 and 0.82 (other methyl protons)	1710 (ϵ-lactone carbonyl)	-	"

Table - I(Contd.)

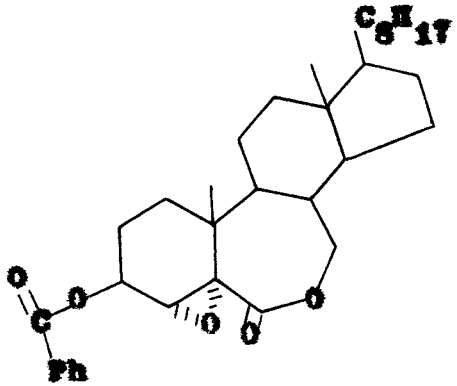
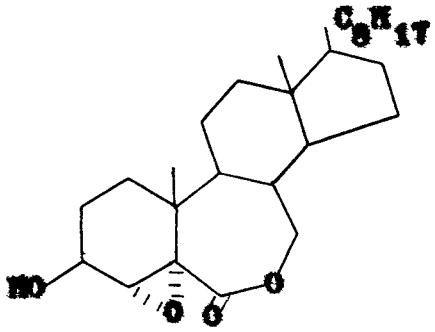
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (XLII)	8.2m (C2-$\underline{H}$, C6-$\underline{H}$ of the aromatic ring), 7.66m (C3-$\underline{H}$, C4-$\underline{H}$, C5-$\underline{H}$ of aromatic ring), 5.0br ($W\frac{1}{2}$ 16 Hz, C3-$\prec\underline{H}$), 4.2 mc (C7a-$\underline{H}_2$), 3.3d(C4-$\underline{H}$, J 9 Hz), 0.95s (C10-$C\underline{H}_3$), 0.73s (C13-$C\underline{H}_3$),0.92 and 0.81 (other methyl protons)	1725 (Benzoyloxy and ϵ -lactone carbonyls), 1610, 1595 (C = C, aromatic) and 860 (epoxy)	-	14
 (XLIII)	4.2mc(C7a-$\underline{H}_2$), 3.7br ($W\frac{1}{2}$ 16 Hz, C3-$\prec\underline{H}$), 3.2d (C4-$\underline{H}$, J 8 Hz), 0.93s (C10-$C\underline{H}_3$), 0.71s (C13-$C\underline{H}_3$), 0.85 and 0.77 (other methyl protons)	3460(OH), 1710 (ϵ -lactone carbonyl) and 860 (epoxy)	-	"

Table - I(Contd.)

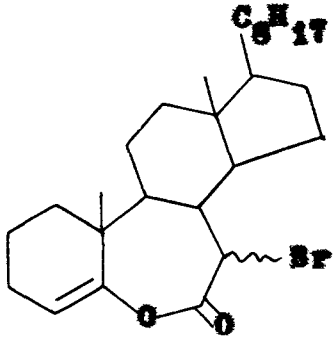
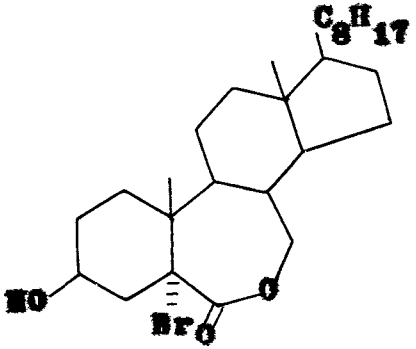
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (XXXIX)	5.8t (C4-$\underline{H}$), 5.2 unc (C7a-$\underline{H}$), 0.99s (C10-$C\underline{H}_3$), 0.72s (C13-$C\underline{H}_3$), 0.93 and 0.85 (other methyl protons)	1766 (enol lactone carbonyl) and 1660 (C=C-O-)	-	14
 (XLIV)	4.2 unc (C7a-$\underline{H}_2$), 3.78br($W\frac{1}{2}$ 15 Hz, C3-$\underline{H}$), 0.91s (C10-$C\underline{H}_3$), 0.70s (C13-$C\underline{H}_3$), 0.84 and 0.83 (other methyl protons)	3460(OH) and 1720 (ϵ-lactone carbonyl)	-	"

Table - I(Contd.)

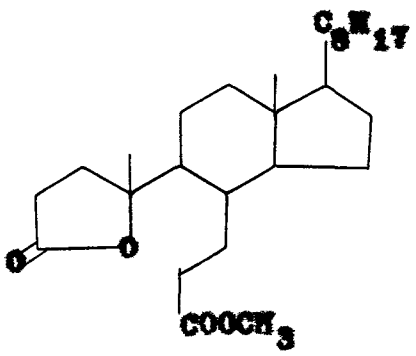
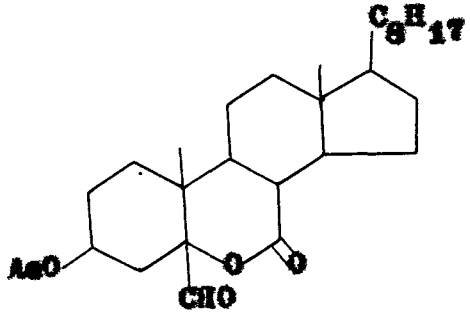
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (LXXXV)	3.63s ($COOCH_3$), 2.36m ($C2-H_2$, $CH_2-COOCH_3$), 1.32s ($C10-CH_3$)	1762 and 1734 (γ-lactone and methyl ester carbonyls)	-	33
 (LXXVIII)	9.62s (CHO), 5.1br ($W\frac{1}{2}$ 14 Hz, C3α-H), 2.01s ($OCOCH_3$), 1.23s ($C10-CH_3$) and 0.67s ($C13-CH_3$)	1724, shoulder at 1748 and 1708 (δ-lactone, acetate and aldehyde carbonyls)	-	34

Table - I(Contd.)

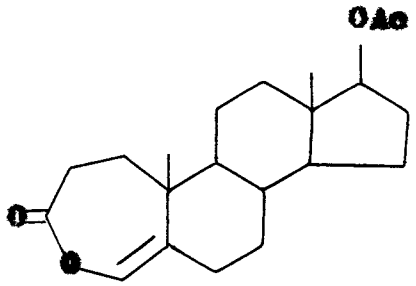
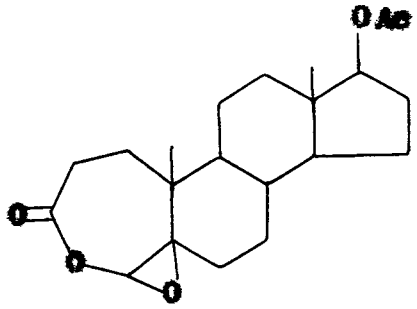
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm(log ϵ)	Ref.
 (LXX)	6.05s(C4a-H), 2.61m (C2-H_2), 1.11s (C10-CH_3), 0.81s (C13-CH_3)	1766, 1733 (enol lactone and acetate carbonyls) and 1644 (C=C)	204 (4.0)	31
 (LXXIII)	4.76s(C4a-H), 2.6m (C2-H_2), 1.18s (C10-CH_3), 0.81s (C13-CH_3)	1773 and 1739 (ϵ-lactone and acetate carbonyls)	-	"

Table - I(Contd.)

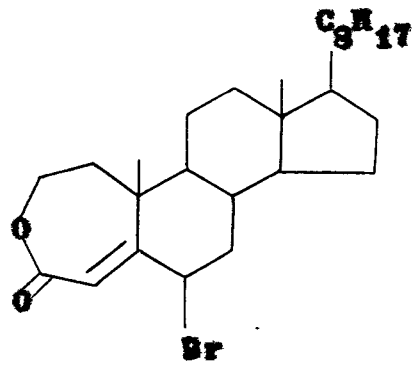
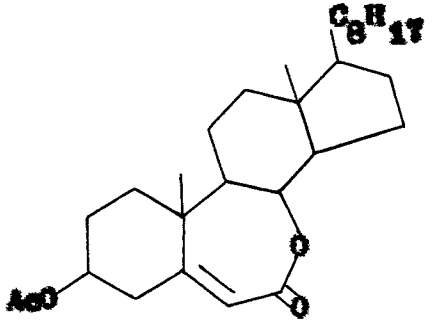
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ∈)	Ref.
 (LXXXIV)	6.0s(C4α-H), 4.9m(C2-H_2), 3.7m (Br-C6-H), 1.6s (C10-CH_3) and 0.77s (C13-CH_3)	1687(C=C-CO-O), 1606 (C=C) and 690 (C-Br)	252 (3.98)	33
 (LXXXVII)	5.9s(C6-H), 4.7br($W\frac{1}{2}$ 12 Hz, C3-αH), 4.27dd (CO-O-C3-H), 1.31s (C10-CH_3), 0.67s(C13-CH_3)	1737 (CH_3-CO-O) 1690 (C=C-CO-O)	236 (4.02)	34

Table - I(Contd.)

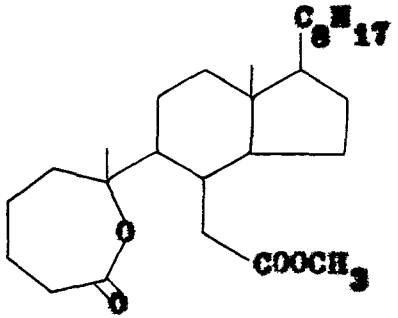
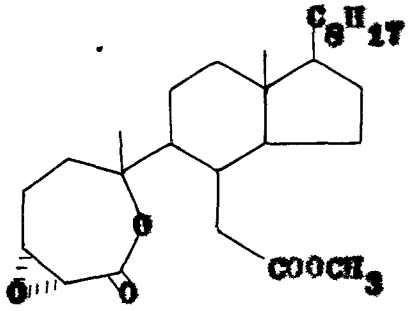
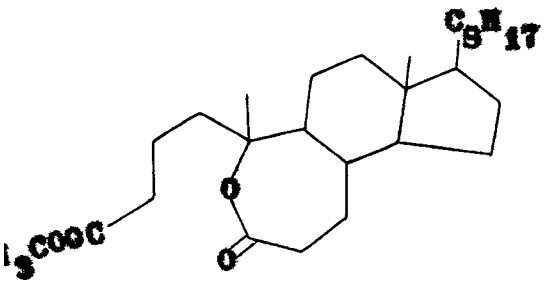
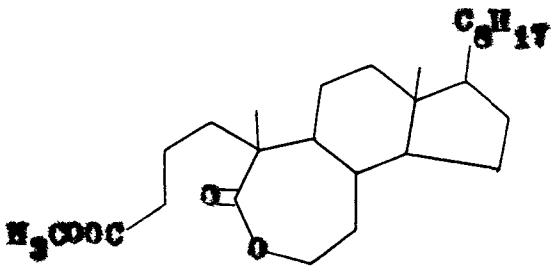
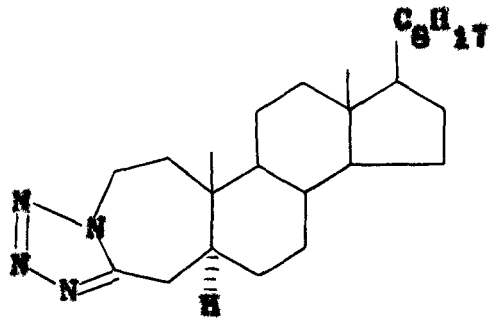
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm(log ϵ)	Ref.
 (CXI)	3.57s ($COOCH_3$), 3.7br ($C4-H_2$, $C7-H_2$), 1.28s ($C10-CH_3$), 0.80s ($C13-CH_3$), 0.88 and 0.86 (other methyl protons)	1740 and 1720 (methyl ester and ϵ-lactone carbonyls)	-	37
 (CXVII)	3.58s($COOCH_3$), 3.3m (C3-H, and C4-H), 2.3br ($C7-H_2$), 1.3s ($C10-CH_3$), 0.7s($C13-CH_3$), 0.92 and 0.81 (other methyl protons)	1740 and 1718 (methyl ester and ϵ-lactone carbonyls)	-	"

Table - I(Contd.)

Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (CXIII)	3.6s($COOCH_3$), 2.28br (C3-H_2, C7-H_2), 1.28s (C10-CH_3), 0.7s (C13-CH_3), 0.96 and 0.84 (other methyl protons).	1740 and 1720 (methyl ester and ϵ-lactone carbonyls)	-	37
 (CXIV)	3.84m($CO-OCH_2$), 3.55s ($COOCH_3$), 2.2br(C3-H_2), 1.06s (C10-CH_3), 0.68s (C13-CH_3), 0.98 and 0.82 (other methyl protons)	1742 and 1720 (methyl ester and ϵ-lactone carbonyls)	-	"

Tetrazolosteroids

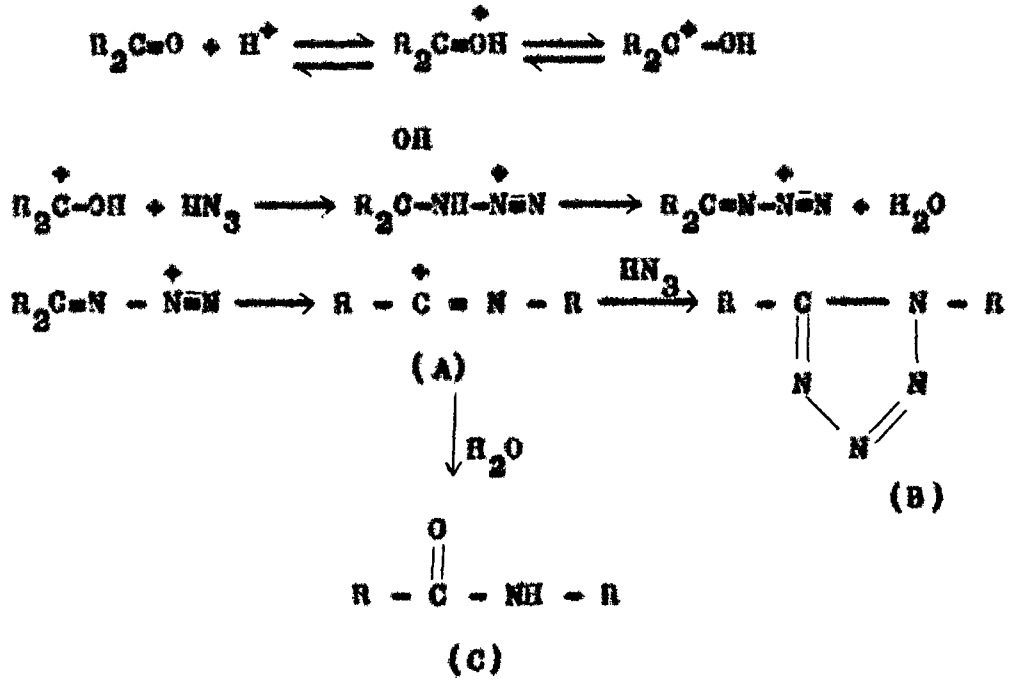
When the five membered doubly unsaturated heterocycle with one carbon and four nitrogen atoms is fused with steroid framework it comes to be known as tetrazolosteroid e.g. (CXVIII).



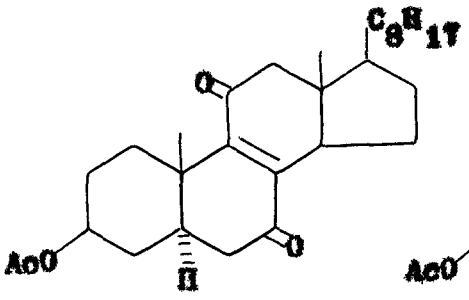
(CXVIII)

The first tetrazole was recognized in 1885 by Bladin[39] during an investigation of dicyanophenylhydrazine. An excellent review, covering methods of synthesis of tetrazoles and almost every conceivable aspect of tetrazole chemistry, is given by Benson[39]. One of the most valuable methods for the preparation of tetrazoles is the rearrangement reaction between ketones and an excess of hydrazoic acid in the presence of strong acids, a modification of the Schmidt reaction[40]. Smith[41] has given a probable mechanism for this transformation. Upon reacting with one mole of the hydrazoic acid, the ketone is converted to the intermediate imidocarbonium ion (A) which then reacts with the second mole of

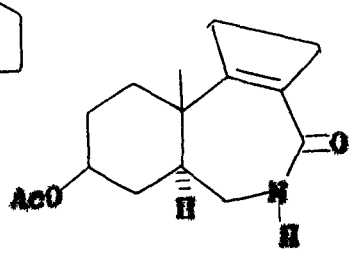
hydrazoic acid to form the tetrazole (B). Combination of hydrazoic acid with the imidocarbonium ion to form a tetrazole (B) competes with reaction of the imidocarbonium ion with water to form an N-substituted amide (C). This mechanism accounts satisfactorily for the necessity of using strong acids as catalyst.



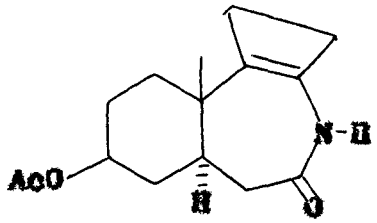
Probably the first example of the formation of a tetrazole in steroid and triterpenoid field was given by Barnes et al.[42] in 1953, who treated 7,11-dioxolanost-8-en-3β-ylacetate (CXIX) with hydrazoic acid and obtained two isomeric monolactams (CXX), (CXXI) and a tetrazole which was considered to have been formed by reaction with the 7-oxo function having the structure (CXXII) or (CXXIII). The structure of the tetrazole could not be firmly established.



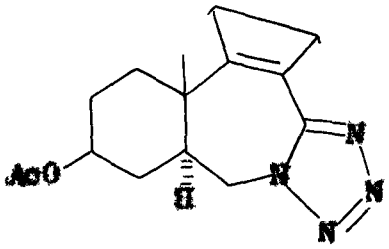
(CXIX)



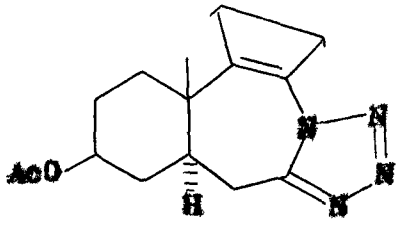
(CXX)



(CXXI)

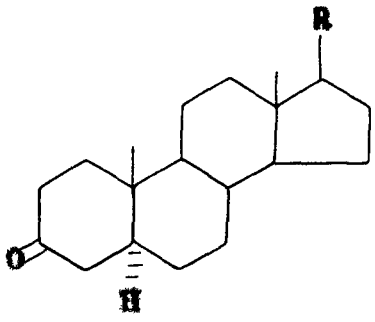


(CXXII)

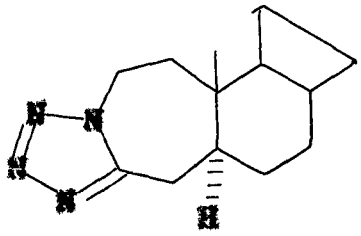


(CXXIII)

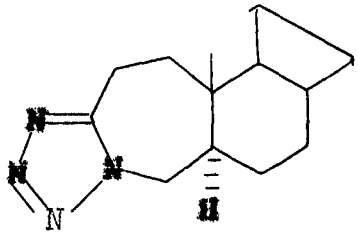
Synthetic chemists did not pay much attention to steroidal tetrazoles until 1968, when Mechoulam[43] reported the synthesis of a number of ring A fused steroidal tetrazoles and claimed that some of them possessed antifertility and antispermatogenic activity. Mechoulam subjected 5α-cholestan-3-one (III) and 17β-hydroxy-5α-androstan-3-one (CXXIV) to Schmidt reaction using excess of hydrazoic acid which gave a mixture of isomeric tetrazoles (CXVIII, CXXV) and (CXXVI, CXXVII), respectively, containing 3-aza-A-homo-(3,4-d) tetrazole and 4-aza-A-homo- (3,4-d) tetrazole systems.



(III) R, C_8H_{17}
(CXXIV) R, OH

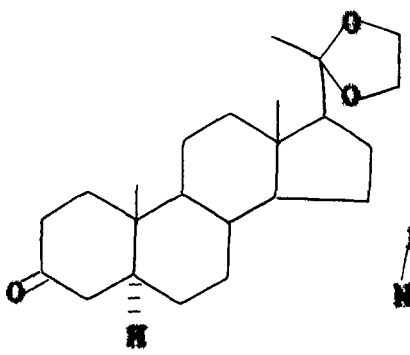


(CXVIII) R, C_8H_{17}
(CXXVI) R, OH

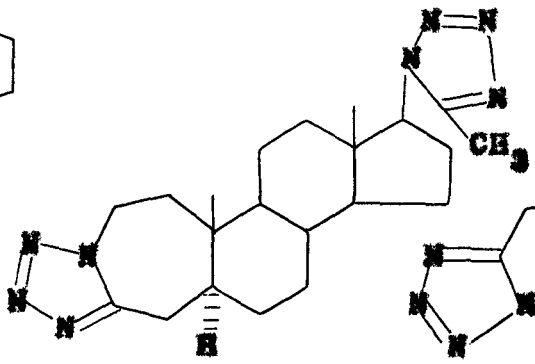


(CXXV) R, C_8H_{17}
(CXXVII) R, OH

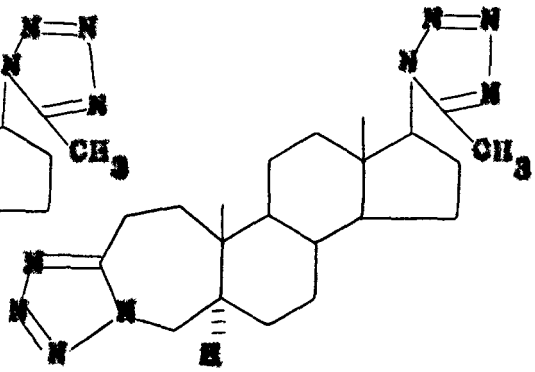
Similar treatment of 20,20-ethylenedioxy-5α-pregnan-3-one (CXVIII) furnished a mixture of 17β-(5-methyl tetrazole-1-yl)-3-aza-A-homo-5α-androstano(3,4-d) tetrazole (CXXIX) and its 4-aza isomer (CXXX). Under the experimental conditions acetal ring at C20 hydrolysed to C20 ketone which further reacted with hydrazoic acid to form the tetrazole at 17-position.



(CXXVIII)

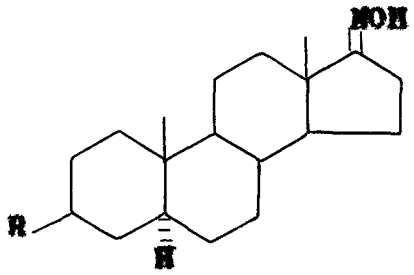


(CXXIX)

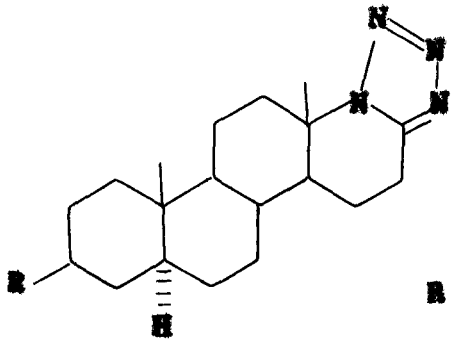


(CXXX)

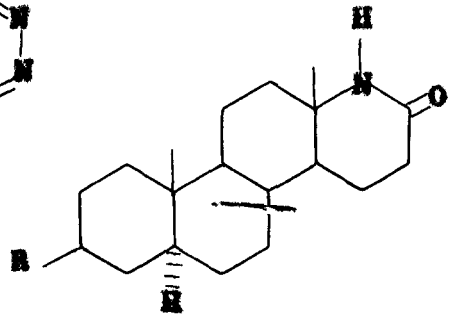
Once the pharmacological potential of steroidal tetrazoles was realized, spate of attempts were made towards their synthesis and subsequently several papers appeared concerning their synthesis and biological activity. Cervantes et al.[44] of Syntex group reported the formation of ring D fused tetrazoles from the reaction of 17-ketoximes with an excess of sodium azide in the presence of sulphuric acid. 17-Hydroximino-5α-androstan (CXXXI) afforded 17a-aza-D-homo-5 -androstano (17a,17-d) tetrazole (CXXXII) and the D-homolactam (CXXXIII). Similarly, the oxime (CXXXIV) yielded 3β-acetoxy-17a-aza-D-homo-5α-androstano (17a,17-d) tetrazole(CXXXV) and the lactam (CXXXVI) and the oxime (CXXXVII) gave 17a-aza-3-hydroxy-D-homoestra-1,3,5(10)-trieno(17a,17-d) tetrazole 3-methyl ether (CXXXVIII) along with the seconitrile (CXXXIX) and the(CXL).



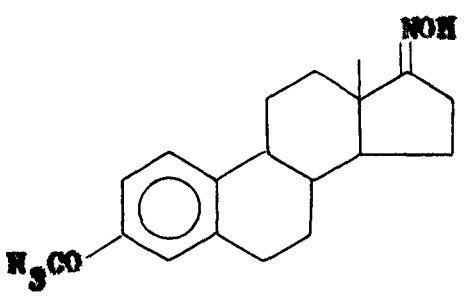
(CXXI) R,H
(CXXXIV) R,OAc



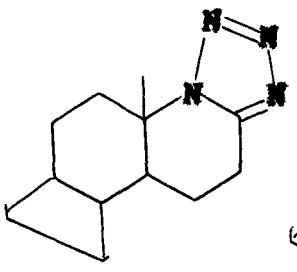
(CXXXII) R,H
(CXXXV) R,OAc



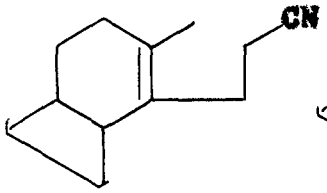
(CXXXIII) R,H
(CXXXVI) R,OAc



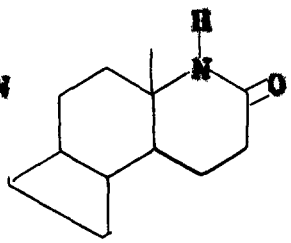
(CXXXVII)



(CXXXVIII)

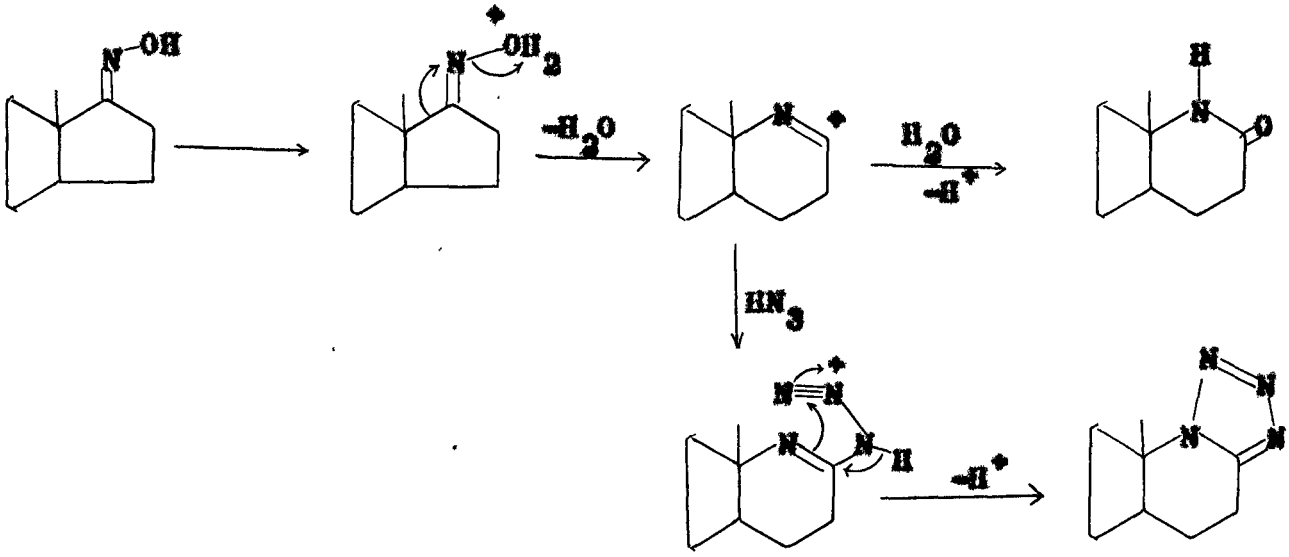


(CXXXIX)

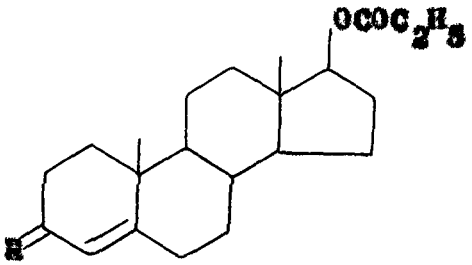


(CXL)

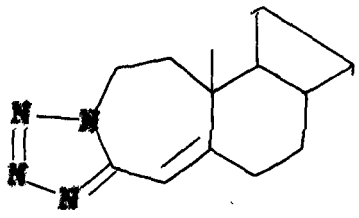
Cervantes et al.[44] proposed that both the tetrazoles and the lactams are a result of the action of hydrazoic acid on an imidocarbonium ion intermediate formed by rearrangement of the oxime. Lactams were shown not to be the precursors of tetrazoles because the lactams were recovered unchanged in an attempted reaction with hydrazoic acid.



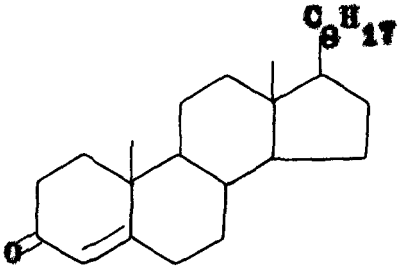
In 1970, Mourai and Syhora[45] reported the synthesis of a number of 3-aza-A-homo-4a-eno (3,4-d) tetrazole analogues from the corresponding 3-oxo-4-enosteroids from their reaction with hydrazoic acid. 3-Oxoandrost-4-en-17β-propionate (LIV) under reaction conditions gave the corresponding tetrazole(CXLI). The tetrazole (CXLI) was also obtained when 3 -hydroximino-androst-4-en-17β-propionate (CXLII) was treated with hydrazoic acid. Similarly, 3-oxocholest-4-ene (LI) furnished the corresponding tetrazole (CXLIII).



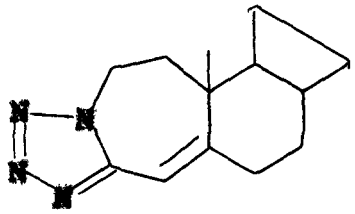
(LIV) R=O
(CXLII) R=NOH



(CXLI)

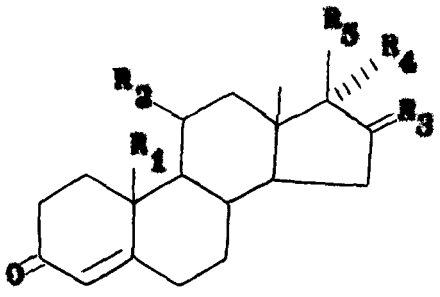


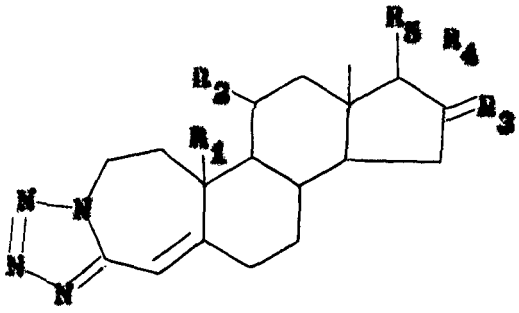
(LI)



(CXLIII)

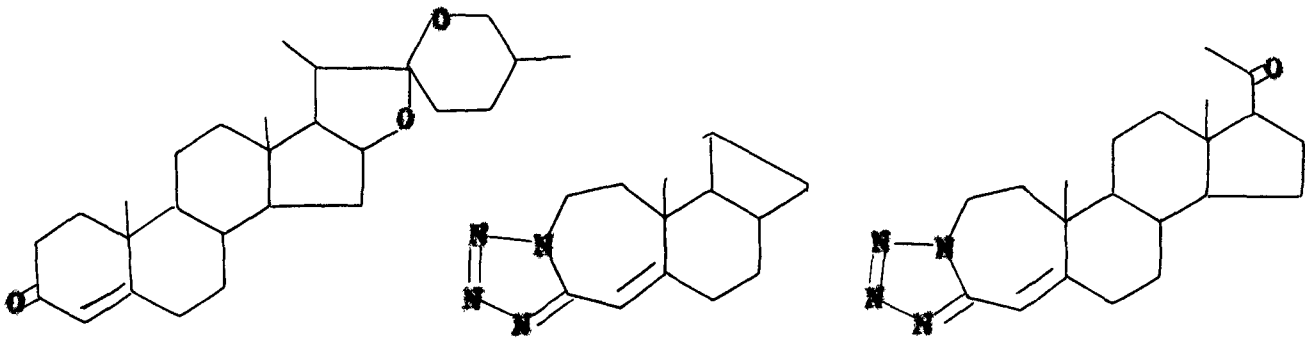
They synthesized a number of totrazoles from 3-oxo-4-enosteroids varying from each other with respect to substituents. These are tabulated below. Some of them were found to exhibit biological activity of one kind or the other.





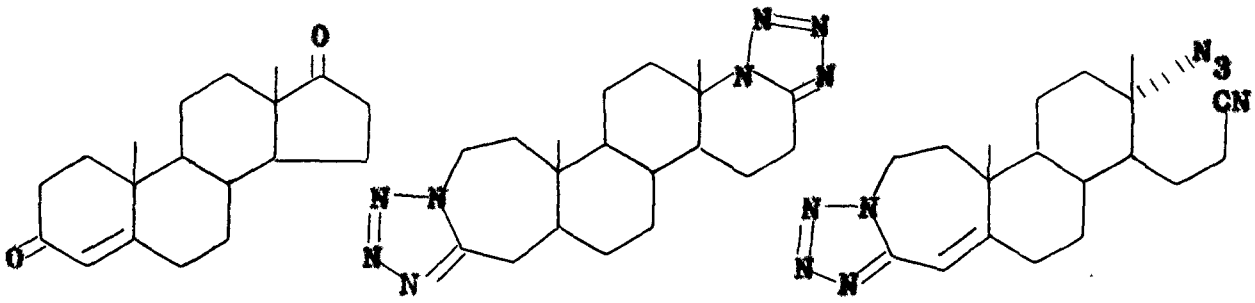
Starting compound	R_1	R_2	R_3	R_4	R_5	Additional substituent	Product
(CXLIV)	H	H	H_2	H	$OCO(CH_2)_2C_6H_5$	-	(CXLV)
(CXLVI)	"	"	"	"	"	1α-CH_3	(CXLVII)
(CXLVIII)	CH_3	"	"	"	OH	-	(CXLIX)
(LXVIII)	"	"	"	"	$OCOCH_3$	-	(CL)
(CLI)	"	"	"	"	$OCO(CH_2)_2C_6H_5$	-	(CLII)
(CLIII)	CH_3	H	H_2	$CH=CH_2$	OH	-	(CLIV)
(CLV)	"	"	"	C≡CH	"	-	(CLVI)
(CLVII)	"	"	"	CH_3	"	Δ^6	(CLVIII)
(CLIX)	"	α-OH	"	H	$COCH_3$	-	(CLX)
(CLXI)	"	H	"	$OCOCH_3$	"	6α-CH_3	(CLXII)
(CLXIII)	"	"	16β-CH_3	16,17-epoxy	"	-	(CLXIV)
(CLXV)	"	"	CH_2	OH	"	-	(CLXVI)
(CLXVII)	"	"	"	$OCOCH_3$	"	-	(CLXVIII)
(CLXIX)	"	"	"	"	"	Δ^6	(CLXX)
(CLXXI)	"	"	H_2	H	$COCH_2OCOCH_3$	-	(CLXXII)
(CLXXIII)	"	"	"	OH	"	-	(CLXXIV)
(CLXXV)	"	"	"	"	"	$\Delta^{9(11)}$	(CLXXVI)
(CLXXVII)	"	"	"	"	"	9β,11β-epoxy	(CLXXVIII)
(CLXXIX)	"	=O	"	"	"	$COCH_2OH$	(CLXXX)
(CLXXXI)	"	"	"	"	"	$COCH_2OCOCH_3$	(CLXXXII)
(CLXXXIII)	"	β-OH	H_2	"	"	9α-F	(CLXXXIV)

With the interest of obtaining tetrazolosteroids capable of affecting the nervous system, Singh et al.[46] treated (25R)-spirost-4-en-3-one (CLXXXV) with an excess of hydrazoic acid in the presence of borontrifluoride etherate and obtained a tetrazole which was shown to be 3-aza-A-homo-(25R)-spirost-4a-eno(3,4-d) tetrazole (CLXXXVI). The tetrazole (CLXXXVI) on Marker degradation gave 3-aza-A-homopregna-4a,16-dieno(3,4-d) tetrazole-20-one(CLXXXVII).



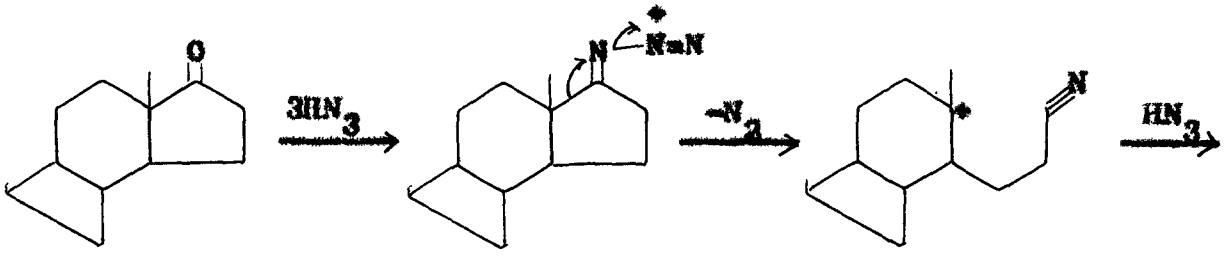
(CLXXXV) (CLXXXVI) (CLXXXVII)

Singh et al.[47,48] have reported that androst-4-ene-3,17-dione (CLXXXVIII) on treatment with hydrazoic acid-borontrifluoride etherate in chloroform yielded the expected 3,17a-diaza-A,D-bishomoandrost-4a-eno(3,4-d)(17a,17-d) bistetrazole (CLXXXIX) and an unusual product, 13,17-seco-13α-azido-A-homoandrost-4a-eno(3,4-d) tetrazole-17-nitrile (CXC). The azido-nitrile function in (CXC) cyclised on heating to give (CLXXXIX). This is claimed to be the first instance of the isolation of an azido-nitrile formed under Schmidt reaction conditions and its thermal cyclisation to a tetrazole.

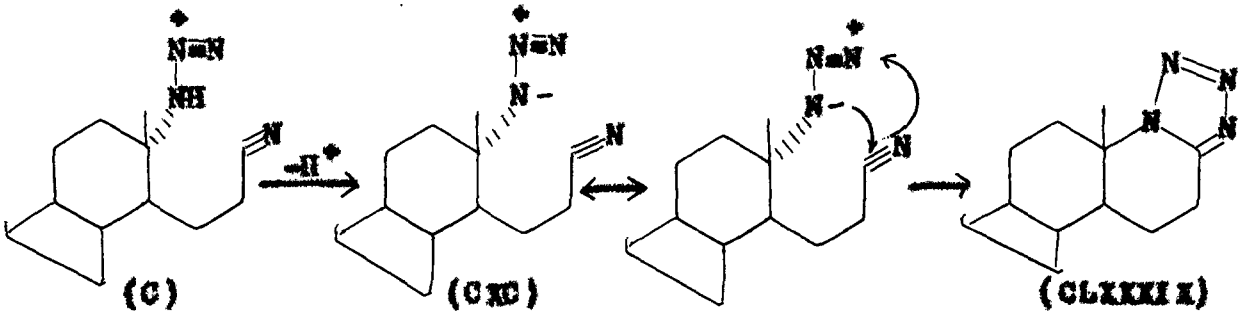


(CLXXXVIII) (CLXXXIX) (CXC)

To account for this novel cleavage of 17-oxosteroid (CLXXXVIII) to azidonitrile (CXC) and its subsequent cyclization to tetrazole (CLXXXIX), the following mechanism was proposed.



(CLXXXVIII) (a) (b)

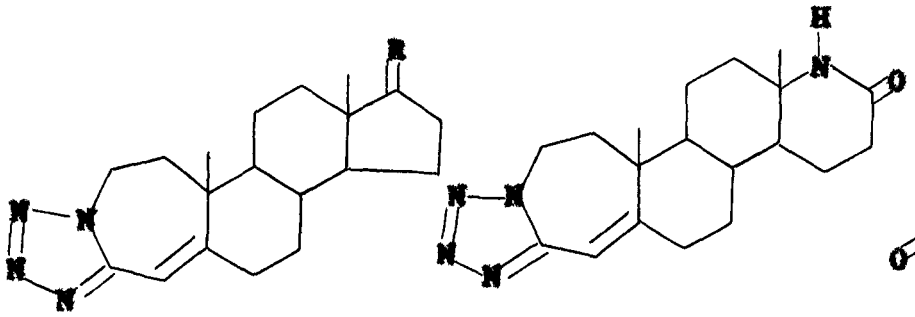


(C) (CXC) (CLXXXIX)

The possibility of an intermediate olefinic entity reacting with hydrazoic acid in the presence of Lewis acid to give the

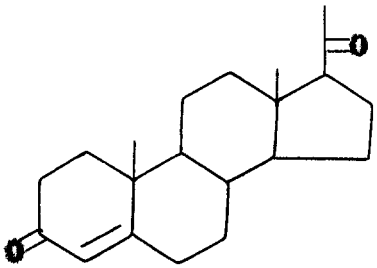
addition product was ruled out in view of the stereospecific nature of the product. The configuration at C13 is retained as evident from thermal cyclization of (CXC) to (CLXXXIX). The mechanism satisfactorily accounts for the observed stereo-specificity; the carbonium ion (b) retaining its configuration, and instantly reacting with hydrazoic acid to give (c). However, the alternative concerted reaction involving approach of azide from the backside in (a) leading to (c) cannot be excluded. This kind of reaction is termed as 1,3-dipolar addition by the authors on the argument that for intermolecular reaction without a catalyst, it is particularly necessary that the nitrile function be sufficiently activated by electron withdrawing groups[49].

Singh et al.[50] have reported that the oxime (CXCII) of the ketotetrazole (CXCI) on rearrangement with thionyl chloride afforded 3,17a-diaza-A,D-bishomoandrost-4a-eno(3,4-d) tetrazol-17-one (CXCIII); the structure was confirmed by its preparation from 17a-aza-D-homoandrost-4-ene-3,17-dione (CXCIV) by treatment with an excess of hydrazoic acid in the presence of boron-trifluoride etherate.

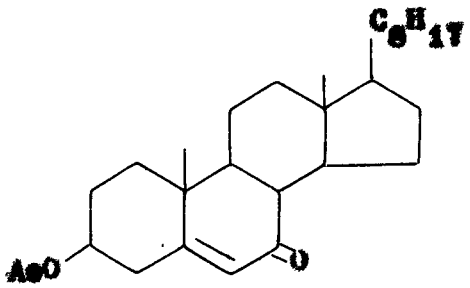


(CXCI) R,O (CXCIII) (CXCIV)
(CXCII) R, NOH

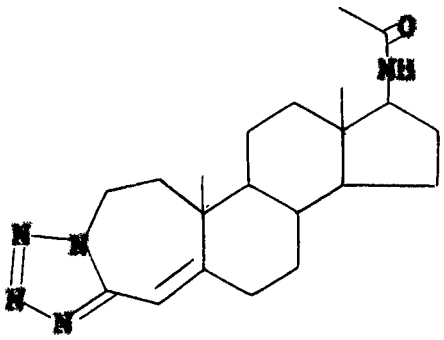
Singh et al.[51] have synthesized tetrazoles from progesterone (CXCV) and 7-oxocholest-5-en-3β-yl acetate (LXXXVI) on treatment with an excess of hydrazoic acid in borontrifluoride etherate. Progesterone (CXCV) was shown to furnish 17β-acetamido-3-aza-A-homoandrost-4a-eno(3,4-d) tetrazole (CXCVI) and 17β-(5-methyl tetrazol-1-yl)-3-aza-A-homoandrost-4a-eno(3,4-d) tetrazole (CXCVII). In order to further confirm the structure of tetrazole (CXCVI) 17β-acetamidoandrost-4-en-3-one (CXCVIII) was treated with hydrazoic acid-borontrifluoride etherate. The product of this reaction was found identical with (CXCVI) in all respects. Under similar conditions, (LXXXVI) afforded 7a-aza-B-homocholest-5-eno(7a,7-d) tetrazole-3β-yl acetate (CXCIX). Base hydrolysis of (CXCIX) yielded 7a-aza-B-homocholest-4-eno(7a,7-d) tetrazol-3β-ol (CCI); a product of allylic shift from C5-C6 to C4-C5. Acylation of (CCI) gave 7a-aza-B-homocholest-4-eno(7a,7-d) tetrazol-3β-yl acetate (CCII). Acid hydrolysis of (CXCIX), however, proceeded without double bond migration giving rise to 7a-aza-B-homocholest-5-eno(7a,7-d) tetrazol-3β-ol (CC) whose acylation regenerated the ester (CXCIX). Both the hydrolytic products (CC) and (CCI), on Oppenauer oxidation yielded the same α,β-unsaturated keto tetrazole, 7a-aza-B-homocholest-4-eno (7a,7-d) tetrazol-3-one (CCIII).



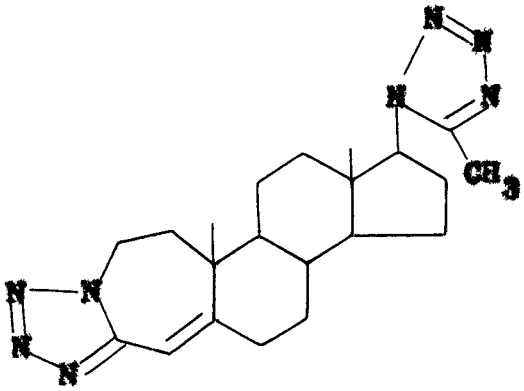
(CXCV)



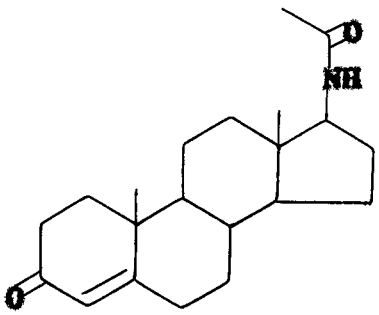
(LXXXVI)



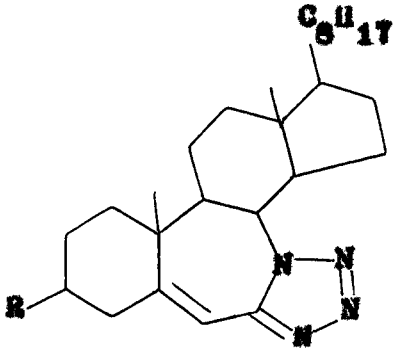
(CXCVI)



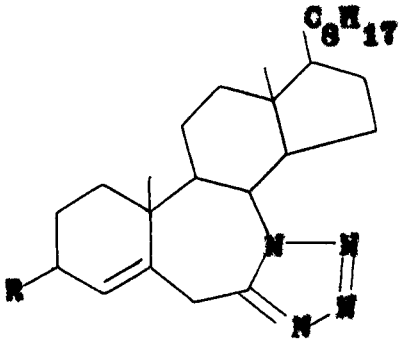
(CXCVII)



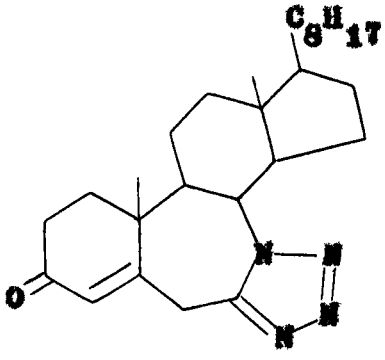
(CXCVIII)



(CXCIX) R, OAc
(CC) R, OH

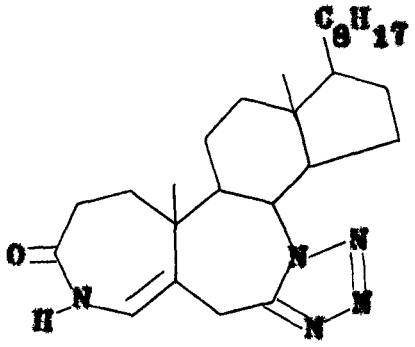


(CCI) R, OH
(CCII) R, OAc

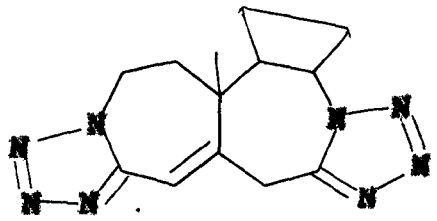


(CCIII)

Later, it was shown by Singh and Malhotra[52] that (CCIII), when treated with one mole equivalent of sodium azide in polyphosphoric acid, afforded, 4,7a-diaza-A,B-bishomocholest-4a-eno (7a,7-d) tetrazol-3-one (CCIV). The treatment of (CCIII) with an excess of hydrazoic acid and borontrifluoride etherate as catalyst gave a bistetrazole (CCV).

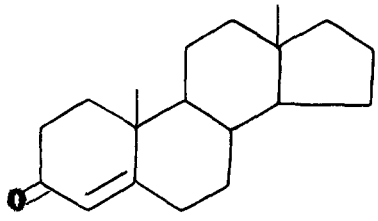


(CCIV)

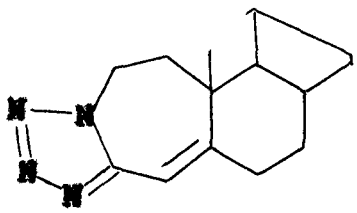


(CCV)

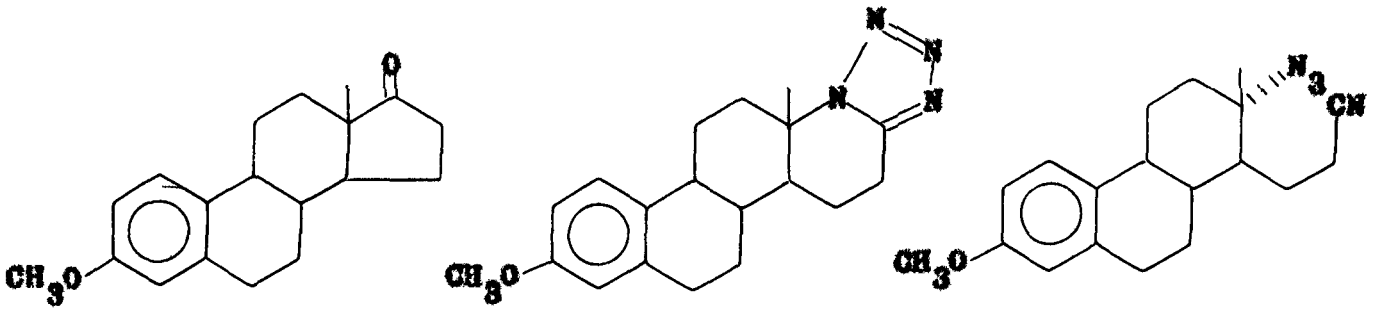
Recently, Singh et al.[53] treated 4-androsten-3-one (CCVI) with an excess of hydrazoic acid in the presence of borontrifluoride etherate and obtained 3-aza-A-homo-4a-androsteno (3,4-d) tetrazole (CCVII), while estrone methyl ether (CCVIII) on similar treatment gave 3-methoxy-17a-aza-D-homo-1,3,5(10)-estratrieno (17a,17-d) tetrazole (CXXXVIII) and 3-methoxy-13,17-seco-13-azido-1,3,5(10)-estratrieno-17-nitrile (CCIX). On heating, the azido-nitrile (CCIX) isomerized to the tetrazole (CXXXVIII).



(CCVI)

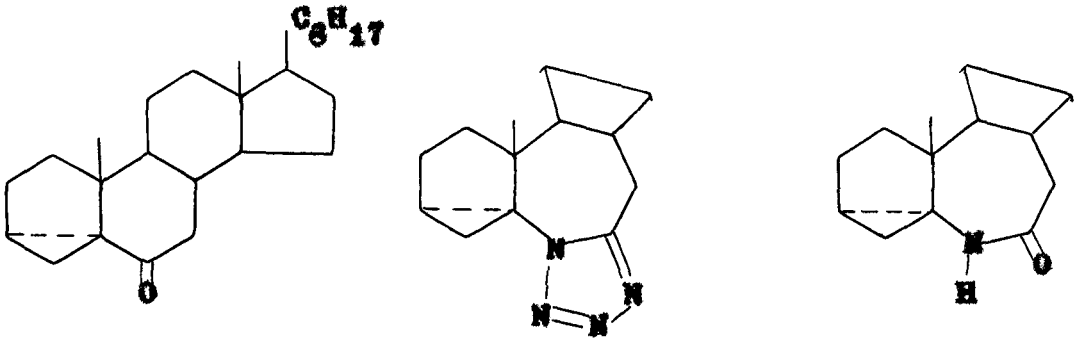


(CCVII)

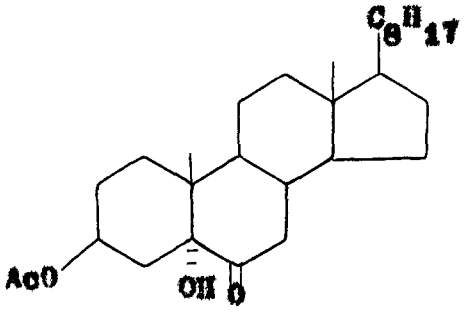


(CCVIII) (CXXXVIII) (CCIX)

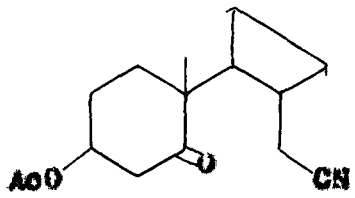
Ahmad et al.[54] have treated 3α,5-cyclo-5α-cholestan-6-one (CCX) with an excess of hydrazoic acid in the presence of borontrifluoride etherate and obtained 6-aza-6-homo-3α,5-cyclo-5α-cholestano (6,7-d) tetrazole (CCXI) and the lactam, 6-aza-6-homo-3α,5-cyclo-5α-cholestan-7-one (CCXII). Under similar reaction conditions, 3β-acetoxy-5-hydroxy-5α-cholestan-6-one (CCXIII) afforded 3β-acetoxy-5-oxo-5,6-secocholestan-6-nitrile (CCXIV).



(CCX) (CCXI) (CCXII)

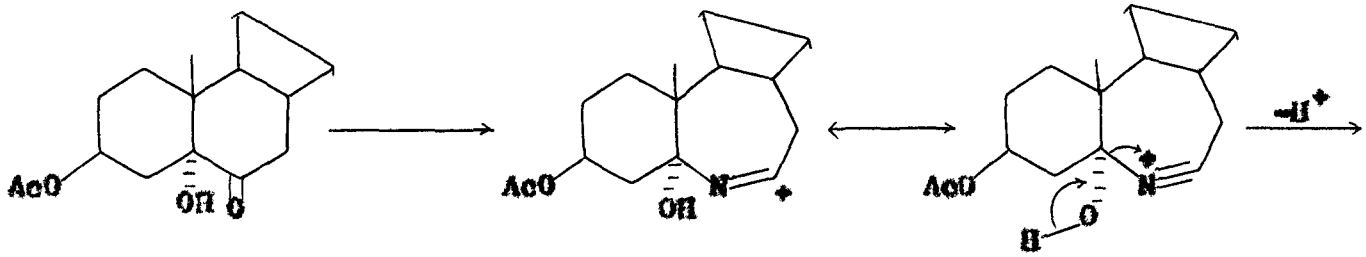


(CCXIII)

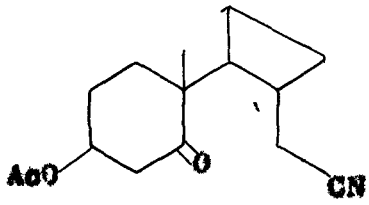


(CCXIV)

The formation of the seco-oxo nitrile (CCXIV) under Schmidt conditions was rationalized in the following manner.



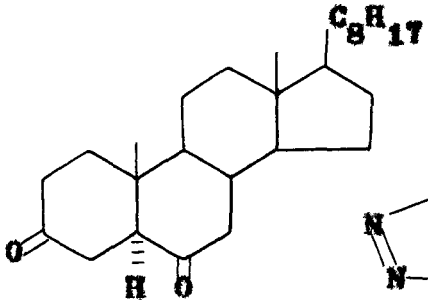
(CCXIII)



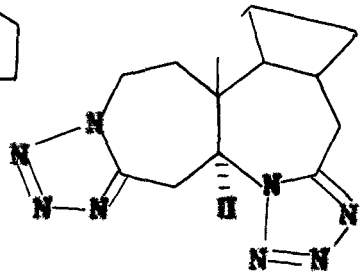
(CCXIV)

Ahmad and coworker[55] treated 5 -cholestane-3,6-dione (CCXV) with an excess of hydrazoic acid in borontrifluoride etherate which afforded a mixture of isomeric bistetrazoles, 3,6-diaza-A,B-bishomo-5α-cholestano (3,4-d)(6,7-d) bistetrazole

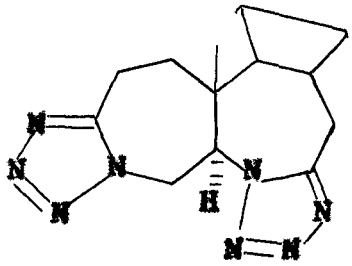
(CCXVI) and 4,6-diaza-A,B-bishomo-5α-cholestano (3,4-d)(6,7-d) bistetrazole (CCXVII). The isomeric bistetrazoles were characterised on the basis of analytical and spectral data. Similar treatment of 5-hydroxy-5α-cholestane-3,6-dione (CCXVIII) furnished 4-aza-A-homo-5-hydroxy-6-oxo-5α-cholestano (3,4-d) tetrazole (CCXIX) and 4-aza-A-homo-3,5a-dioxo-5,6-secocholestano-6-tetrazole (CCXX).



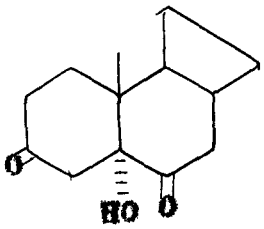
(CCXV)



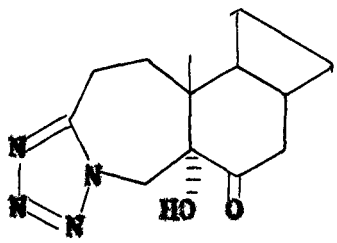
(CCXVI)



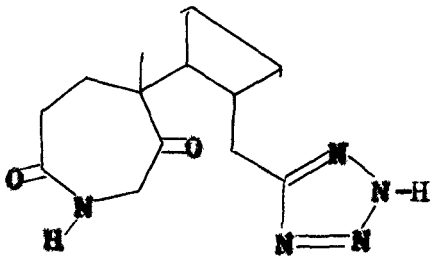
(CCXVII)



(CCXVIII)

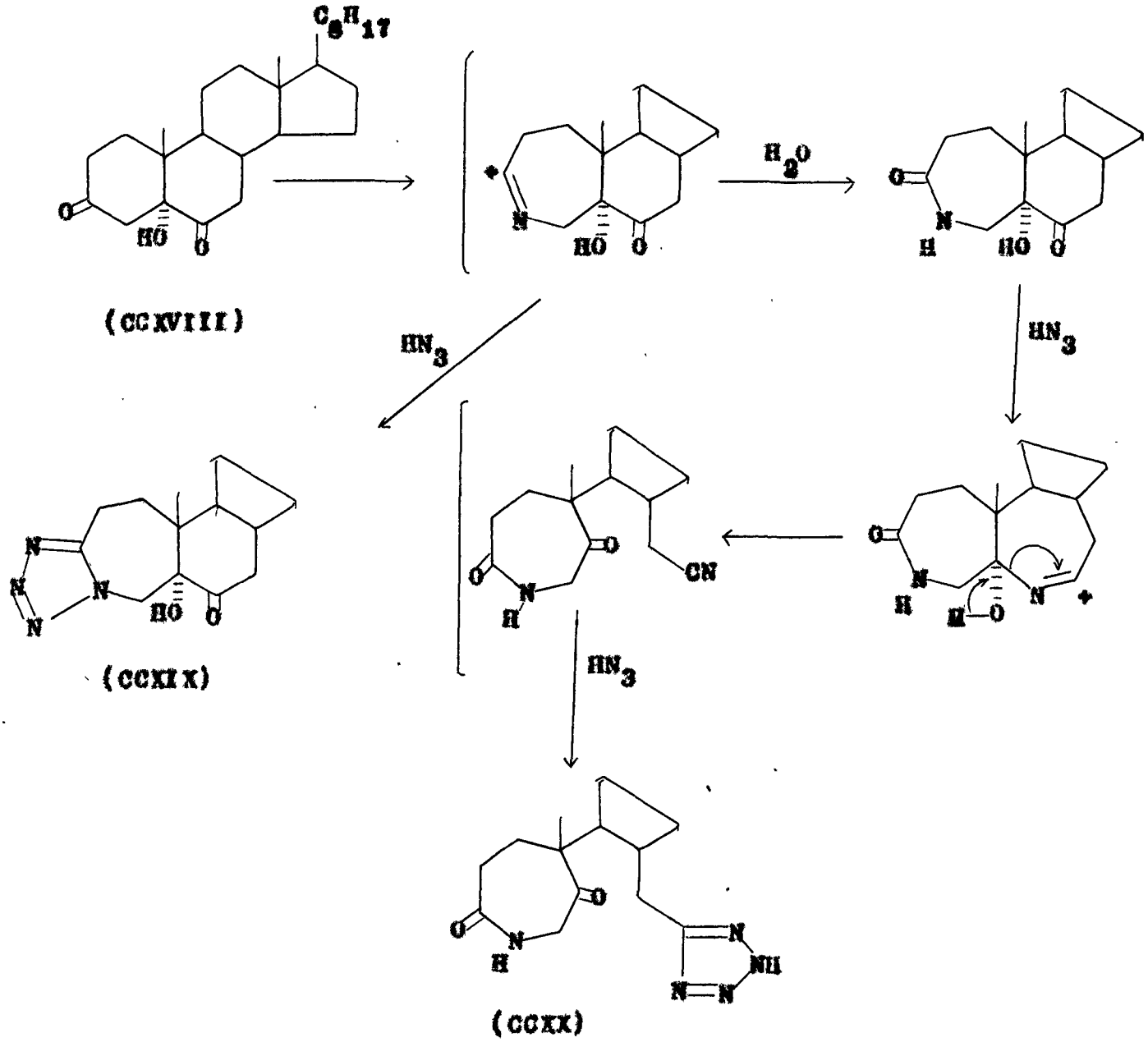


(CCXIX)



(CCXX)

The formation of the lactam tetrazole (CCXX) can be shown to occur according to the following sequence of reactions.

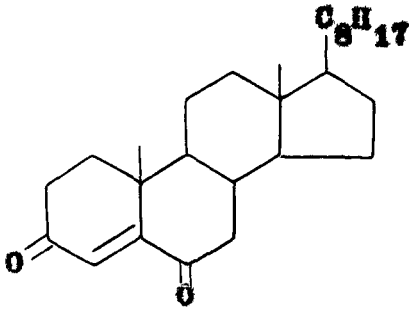


(CCXVIII)

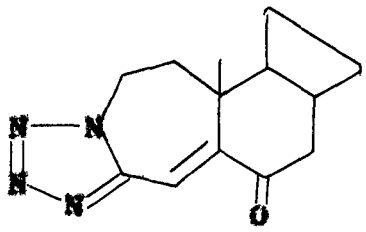
(CCXIX)

(CCXX)

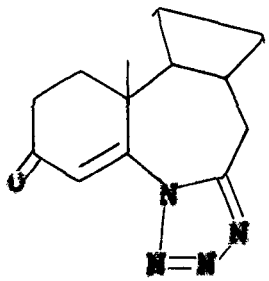
Treatment of cholest-4-ene-3,6-dione (CCXXI) with an excess of hydrazoic acid in borontrifluoride etherate afforded, 3-aza-A-homo-6-oxocholest-4a-eno (3,4-d) tetrazole (CCXXII), 6-aza-B-homo-3-oxocholest-4-eno (6,7-d) tetrazole (CCXXIII) and 3,6-diaza-A,B-bishomocholest-4a-eno (3,4-d)(6,7-d) bistetrazole (CCXXIV)[55].



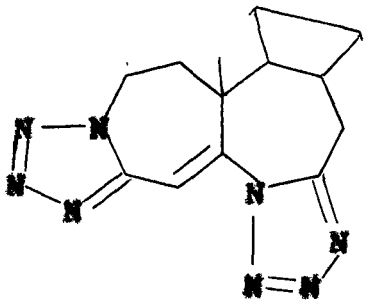
(CCXXI)



(CCXXII)

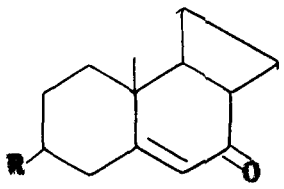


(CCXXIII)

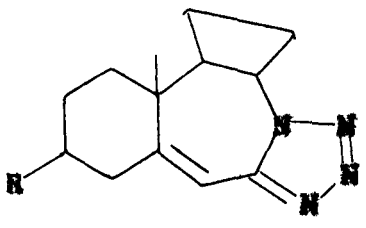


(CCXXIV)

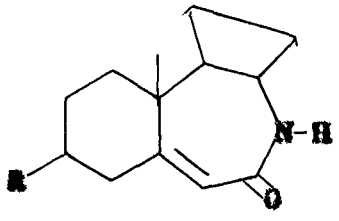
Ahmad et al.[54] treated 3β-chlorocholest-5-en-7-one (CCXXV), with an excess of hydrazoic acid in presence of borontrifluoride etherate which afforded 3β-chloro-7a-aza-B-homocholest-5-eno (7a,7-d) tetrazole (CCXXVI) and the lactam, 3β-chloro-7a-aza-B-homocholest-5-en-7-one (CCXXVII). Cholest-5-en-7-one (XCI) under similar treatment gave tetrazole, 7a-aza-B-homocholest-5-eno (7a,7-d) tetrazole (CCXXVIII) and the lactam 7a-aza-B-homocholest-5-en-7-one (CCXXIX).



(CCXXV) R, Cl
(XCI) R, H

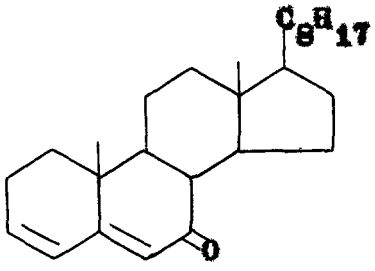


(CCXXVI) R, Cl
(CCXXVIII) R, H

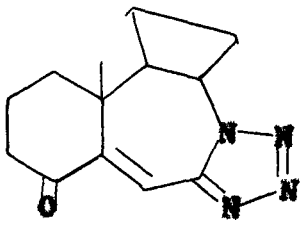


(CCXXVII) R, Cl
(CCXXIX) R, H

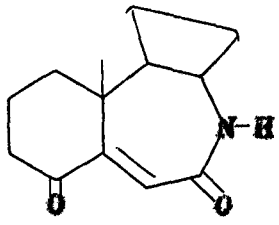
Cholesta-3,5-dien-7-one (CCXXX) with an excess of hydrazoic acid in presence of borontrifluoride etherate gave 7a-aza-B-homo-4-oxocholest-5-eno (7a,7-d) tetrazole (CCXXXI) and 7a-aza-B-homo-4-oxocholest-5-en-7-one (CCXXXII)[54]. Under similar experimental condition, 6β-bromocholest-4-en-3-one (LXXXIII) afforded a bromine free tetrazole, 3-aza-A-homocholesta-4a,6-dieno (3,4-d) tetrazole (CCXXXIII)[55].



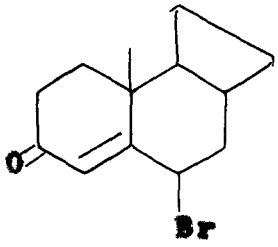
(CCXXX)



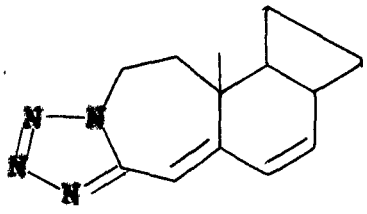
(CCXXXI)



(CCXXXII)



(LXXXIII)



(CCXXXIII)

They proposed the following mechanism for the formation of the tetrazole (CCXXXI) and the lactam (CCXXXII) from (CCXXX) (Scheme - 5).

Scheme - 5

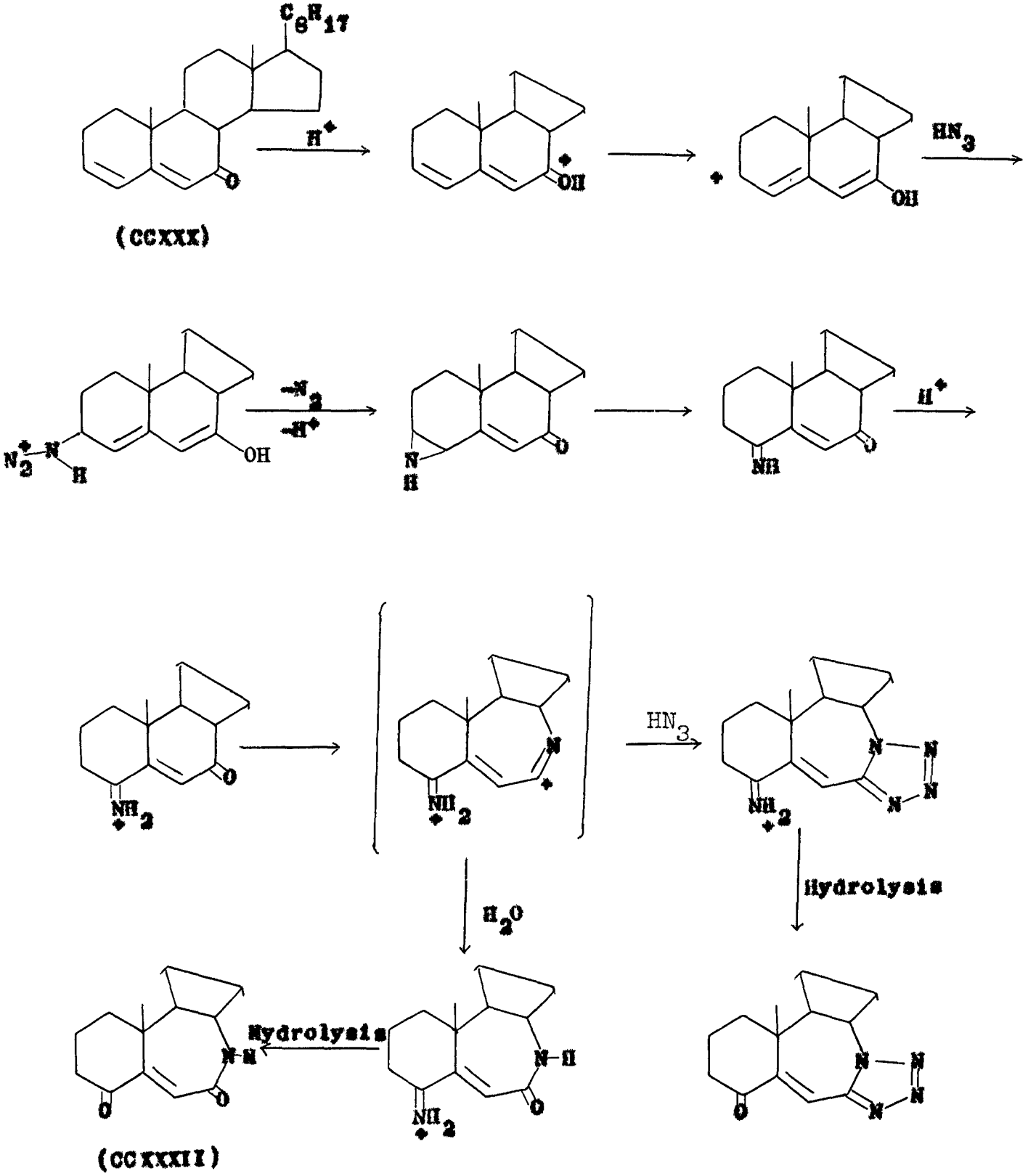


Table - II

Spectral data of some steroidal tetrazoles

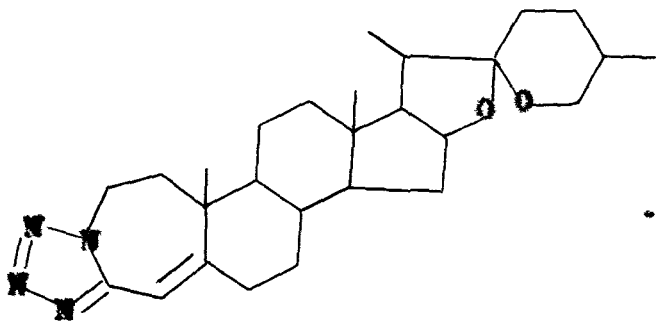
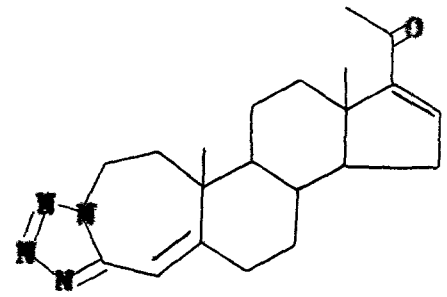
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. mm(log ϵ)	Ref.
 (CLXXXVI)	6.49s (C4a-$\underline{H}$), 4.50m (C2-$\underline{H}_2$), 1.27s (C10-$C\underline{H}_3$)	1650 (C=C), 1530, 1450, 1390 (C=N, N=N)	243 (4.23)	46
 (CLXXXVII)	6.50s (C4a-$\underline{H}$), 4.50m (C2-$\underline{H}_2$), 1.28s (C10-$C\underline{H}_3$)	1660 (C=C), 1520, 1445 1375 (C=N, N=N)	241 (4.41)	"

Table - II(Contd.)

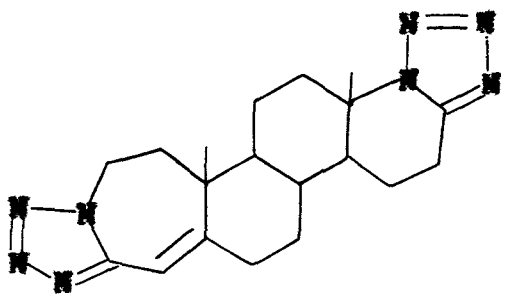
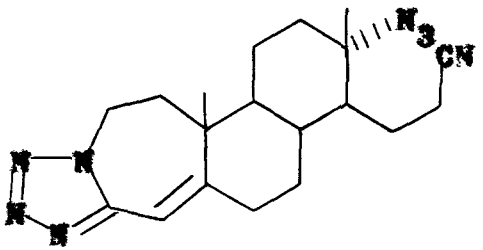
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm(logε)	Ref.
 (CLXXXIX)	6.59s (C4a-H), 4.55m (C2-H_2), 3.0m (C16-H_2), 1.46s (3H), 1.34s (3H)(Angular methyl groups)	1650 (C=C), 1530, 1450 (C=N, N=N)	242 (4.25)	47,48
 (CXC)	6.57s(C4a-H), 4.56m (C2-H_2), 2.49m (NC-C16-H_2), 1.20s (3H), 1.16s (3H)(Angular methyl groups)	2250(CN), 2095(N_3), 1650(C=C), 1530, 1450 (C=N, N=N)	242 (4.23)	,,

Table - II(Contd.)

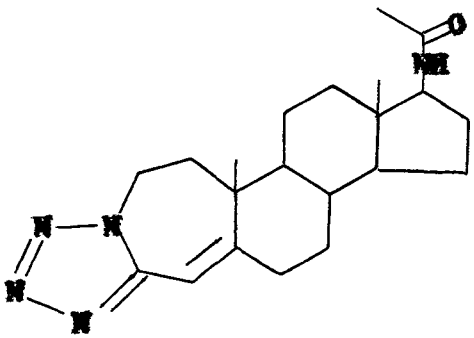
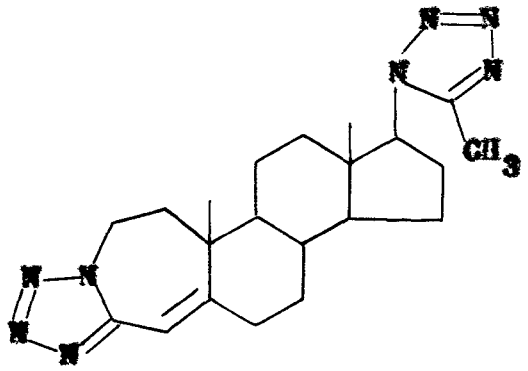
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (CXCVI)	6.50s(C4a-H),4.48m (C2-H_2),3.92m(C17-H), 1.97s (-NHCO-CH_3), 1.24s (C10-CH_3)	3335(NH), 1670(CONH) 1650(C=C), 1530, 1450, 1375 (C=N, N=N)	243 (4.22)	51
 (CXCVII)	6.51s (C4a-H), 4.50m (C2-H_2), 4.15m(C17- H), 2.54s (CH_3-C=N-),1.27s (C10-CH_3),0.80s(C13-CH_3)	1650 (C=C), 1520, 1450, 1390 (C=N, N=N)	243 (4.23)	"

Table - II(Contd.)

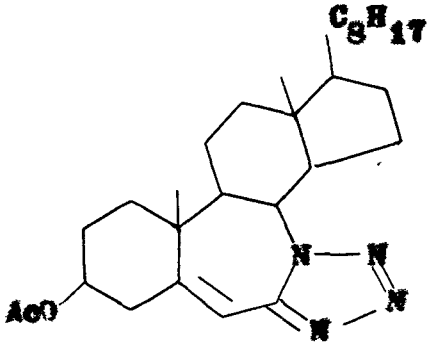
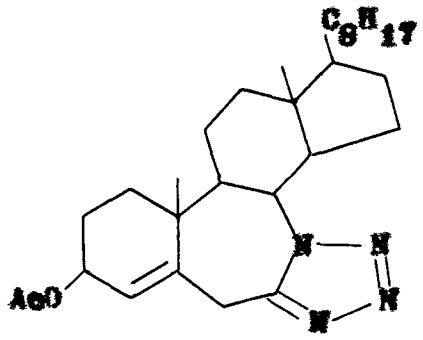
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (CXCIX)	6.62s(C6-$\underline{H}$), 4.75m(C3-$\prec\underline{H}$) 4.25m(C8-$\beta\underline{H}$), 2.05s ($C\underline{H}_3$-COO-), 1.27s(C10-$C\underline{H}_3$) 0.84s (C13-$C\underline{H}_3$)	1737(CH_3-$\underline{C}$O-O), 1665 (C=C), 1505,1465, 1370 (C=N, N=N), 1242 (Acetate)	241 (4.00)	51
 (CCII)	5.63d(C4-$\underline{H}$; J 3.3 Hz), 5.03m (C3-$\prec\underline{H}$), 4.38m (C8-$\beta\underline{H}$), 3.72br (C6-$\underline{H}_2$), 2.03s ($C\underline{H}_3$COO), 1.31s (C10-$C\underline{H}_3$), 0.82d(C13-$C\underline{H}_3$, J 1.3 Hz).	1735 (CH_3-$\underline{C}$O-O), 1530, 1460, 1380, 1370 (C=N, N=N), 1245 (Acetate)	-	,,

Table - II(Contd.)

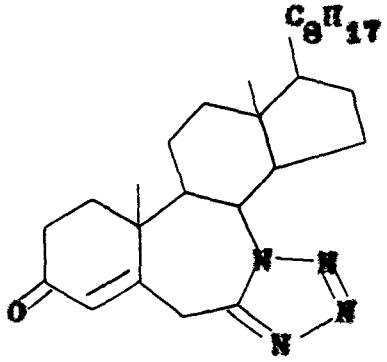
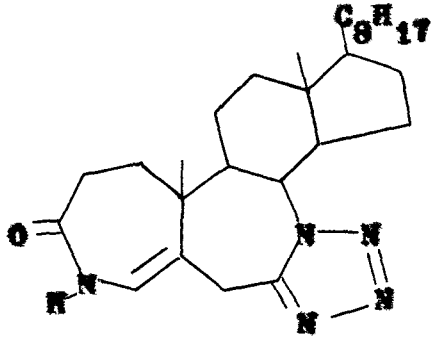
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (CCIII)	5.69s(C4-$\underline{H}$),4.55s(C8-$\beta\underline{H}$), 4.05br(C6-$\underline{H}_2$), 1.17s (C10-$C\underline{H}_3$), 0.83s(C13-$C\underline{H}_3$)	1675(C=C-$\underline{C=O}$), 1625 (C=C), 1530, 1462, 1387 (C=N, N=N)	235 (4.14)	51
 (CCIV)	7.17d(N$\underline{H}$;D_2O exchangeable), 5.81d(C4-$\underline{H}$, J 6 Hz), 4.45m (C8-$\beta\underline{H}$), 3.68br (C6-$\underline{H}_2$), 1.37s(C10-$C\underline{H}_3$), 0.81s (C13-$C\underline{H}_3$).	3225, 3111(NH), 1667 (CONH), 1536, 1460, 1418, 1379 (C=N, N=N)	246 (4.09)	52

Table - II(Contd.)

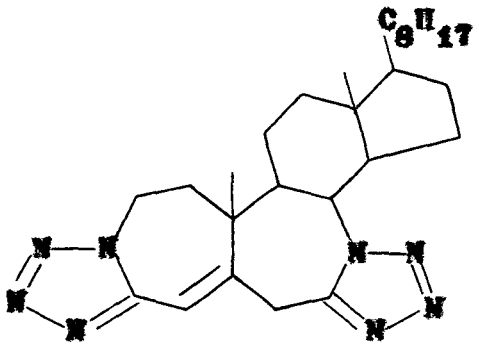
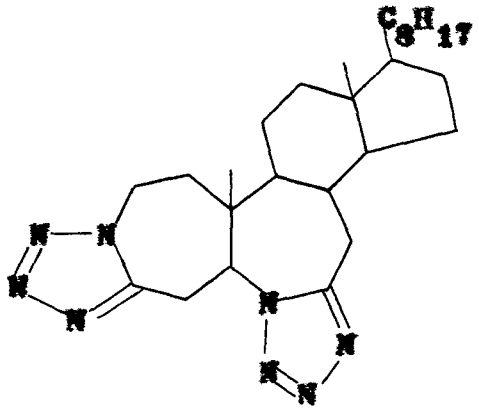
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (log ϵ)	Ref.
 (CCV)	6.68s(C4a-H), 4.56m (C2-H_2, C8-H), 4.04s (C6-H_2), 1.51s(C10-CH_3), 0.85s (C13-CH_3).	1653(C=C), 1527, 1462, 1449, 1439, 1379 (C=N, N=N)	244 (4.20)	52
 (CCXXIV)	7.03s(C4a-H), 4.68mc (C2-H_2), 3.45d(J 14 Hz for one proton of C7a protons) 0.91s (C10-CH_3), 0.73s (C13-CH_3).	1670(C=C), 1530, 1460, 1375(C=N, N=N)	245 (4.11)	54

Table - II(Contd.)

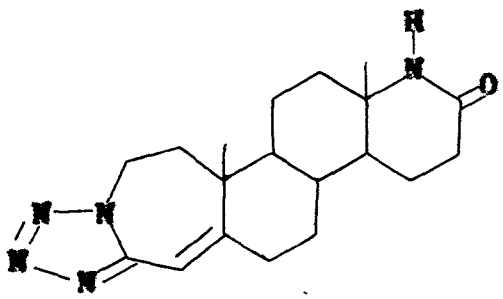
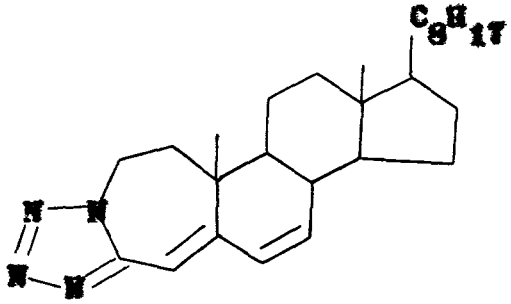
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm(log ϵ)	Ref.
 (CXCIII)	6.50s(C4a-H), 4.50m (C2-H_2), 1.25s(C10-CH_3)	3450, 3125(NH), 1660 (CONH), 1650 (C=C), 1530, 1450, 1335 (C=N, N=N).	240 (4.21)	51
 (CCXXXIII)	6.36s(C4a-H), 6.03mc (C6-H and C7-H), 4.51m (C2-H_2), 1.08s (C10-CH_3), 0.75s (C13-CH_3).	1650(C=C), 1537, 1475, 1390 (C=N, N=N)	287 (4.41)	55

Table -II(Contd.)

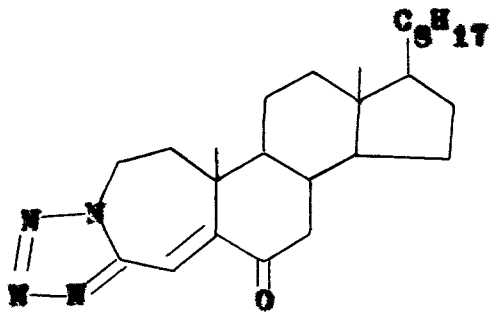
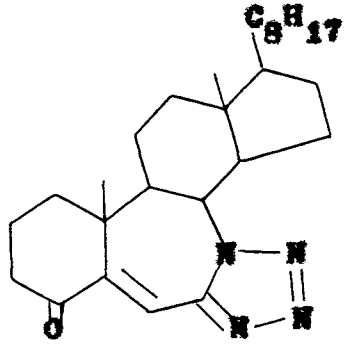
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm (logϵ)	Ref.
 (CCXXII)	7.13s(C4a-H), 4.56m (C2-H_2), 1.11s(C10-CH_3), 0.75s (C13-CH_3).	1690 (C=C-C=O), 1625 (C=C), 1520, 1460,1380(C=N, N=N)	260 (4.66)	55
 (CCXXXI)	7.53s (C6-H), 4.5br (C8-βH), 2.5m(C2-H_2), 1.23s (C10-CH_3), 0.76s (C13-CH_3)	1690 (C=C-C=O), 1630 (C=C), 1500, 1465,1380 (C=N, N=N)	272 (4.42)	54

Table - II(Contd.)

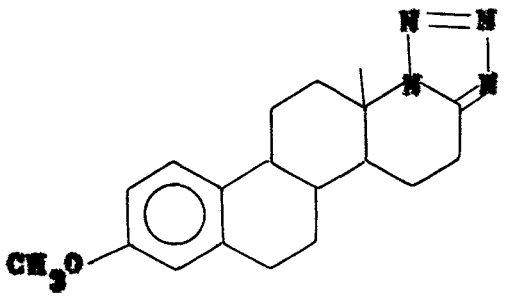
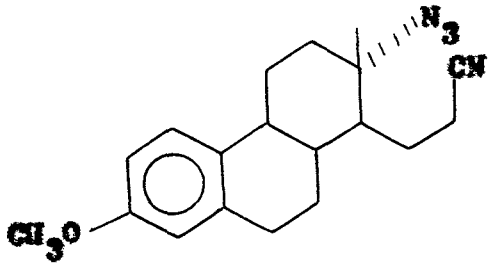
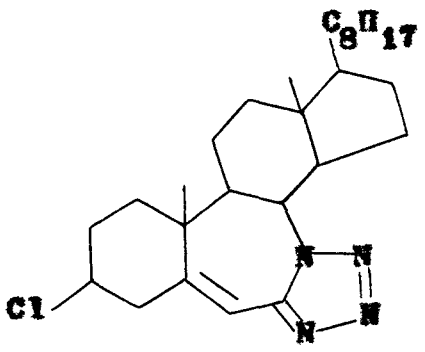
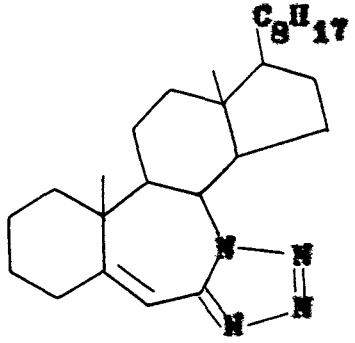
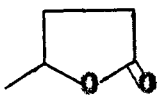
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. mμ(log ε)	Ref.
 (CXXXVIII)	7.1-7.3d(C1-H, J 8 Hz), 6.6-6.8d(C3-H, J 8 Hz), 6.6s (C4-H), 3.75s ($-OCH_3$), 1.4s ($C13-CH_3$)	1580,1500(C=N, N=N)	278,287 (3.21, 3.17)	44
 (CCIX)	6.62-7.30 (C1-H, C2-H, C4-H), 3.75s (OCH_3), 2.90m ($NC-C16-H_2$), 1.21s ($C13-CH_3$).	2252(CN), 2101(N_3), 1515, 1449(C=N, N=N)	275 (3.25)	53

Table - II(Contd.)

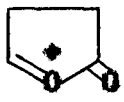
Compound	NMR (δ)	I.R.(cm^{-1})	U.V. nm(log ϵ)	Ref.
 (CCXXVI)	6.63s (C6-$\underline{H}$), 4.21br (C8-$\beta\underline{H}$), 3.81m(C3-$\alpha\underline{H}$), 1.38s (C10-$C\underline{H}_3$), 0.80s (C13-$C\underline{H}_3$)	1660 (C=C), 1505, 1470, 1380 (C=N, N=N), 765 (C-Cl)	240 (4.13)	54
 (CCXXVIII)	6.55s (C6-$\underline{H}$), 4.22br (C8-$\beta\underline{H}$), 1.23s(C10-$C\underline{H}_3$), 0.81s (C13-$C\underline{H}_3$).	1670 (C=C), 1505, 1465,1380 (C=N, N=N)	243 (4.10)	54

Mass Spectrometry of Lactones

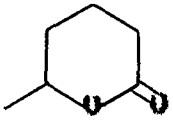
The mass spectral studies on lactones[55-59] have shown that only a small fraction of the total ion current is carried by the molecular ion. The most important peaks arise due to the cleavage of bond adjacent to the ring oxygen atom. The γ - and δ -lactones[57,58] with general formula (CCXXXIV) and (CCXXXV), give fragment ions m/e 85(a) and m/e 99(b), respectively, through this cleavage. This criterion is sometimes used for the distinction[57] of isomeric γ - and δ -lactones. The loss of CO, and CHO moieties from the fragment ion (b) is found to be intense in δ -lactones[58]. The mode of decomposition attributed to the loss of a molecule of CO_2 is insignificant for the lactones of more than 7 or 8 carbon atoms[60].



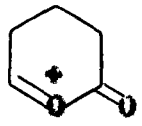
(CCXXXIV)



(a)

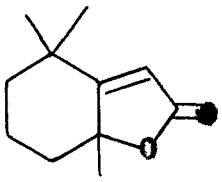


(CCXXXV)

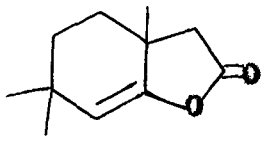


(b)

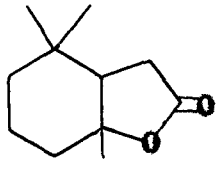
Mass spectra of some bicyclic γ -lactones, such as dihydro actinidiolide (CCXXXVI), 1,4,4-trimethylcyclohexan-2-one acetic acid enol lactone (CCXXXVII) and tetrahydroactinidiolide(CCXXXVIII) were examined by Chen et al.[61] and detailed fragmentation pathways suggested as given in Schemes below.



(CCXXXVI)



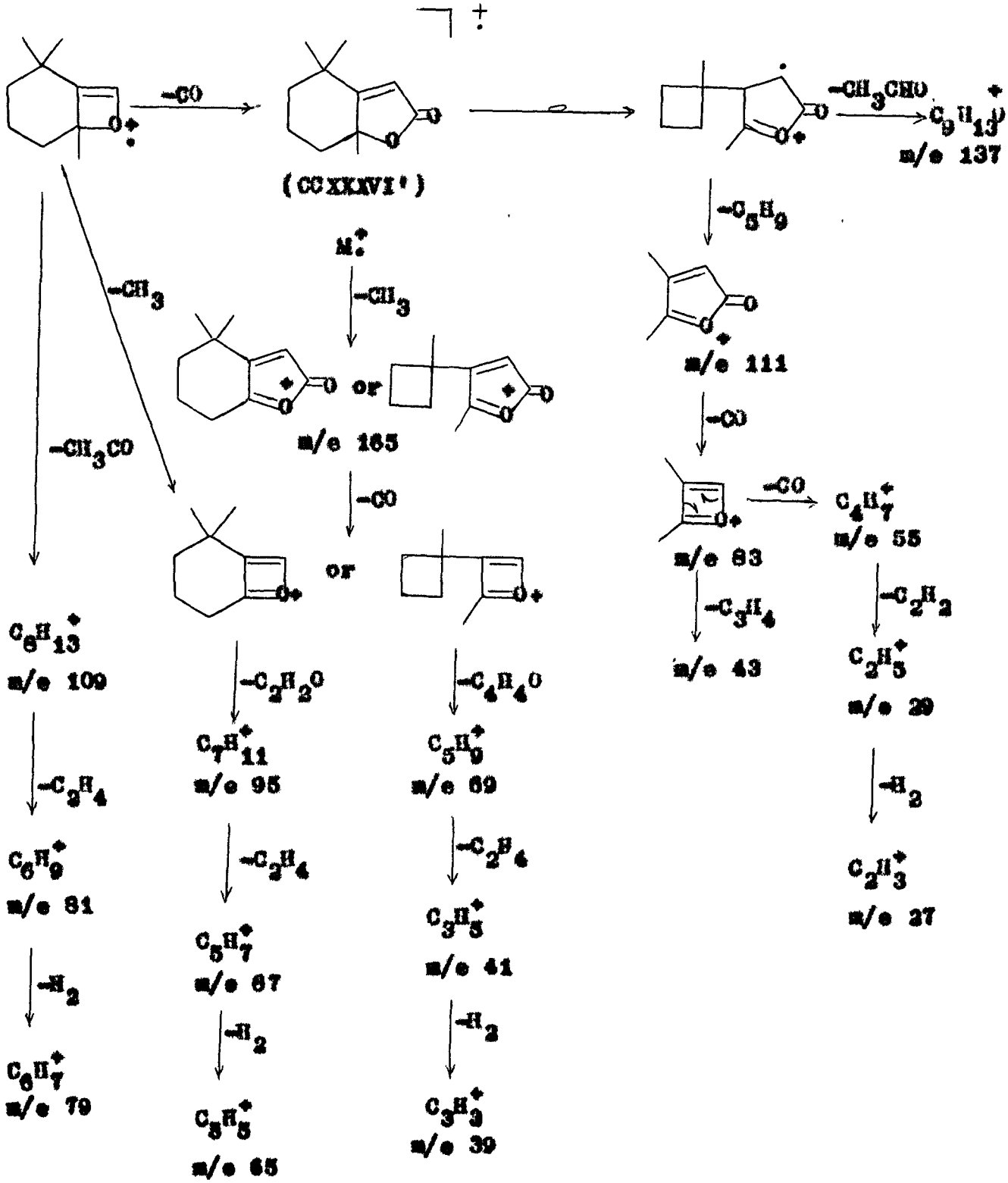
(CCXXXVII)



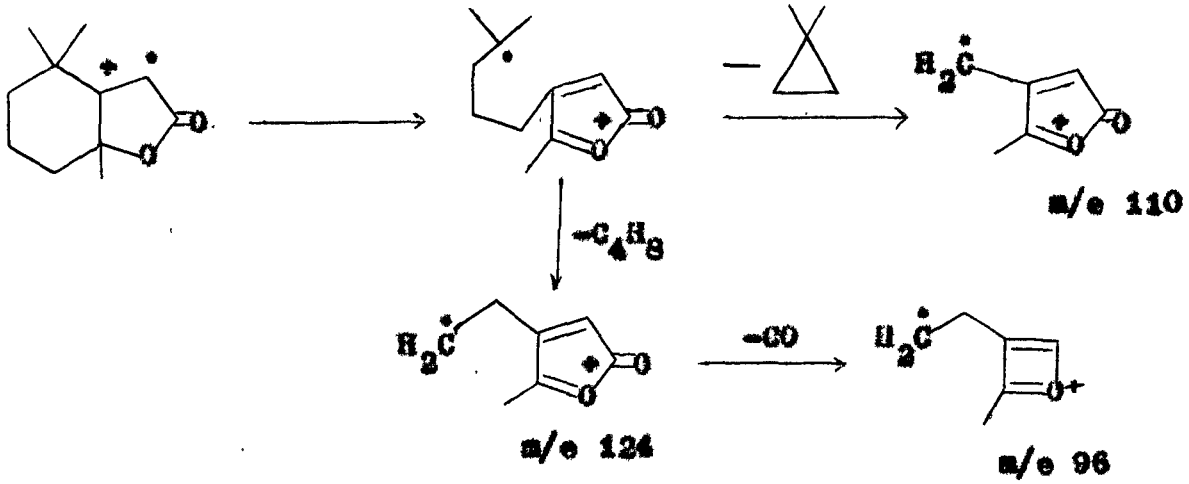
(CCXXXVIII)

Dihydroactinidiolide (CCXXXVI)

Scheme - 6

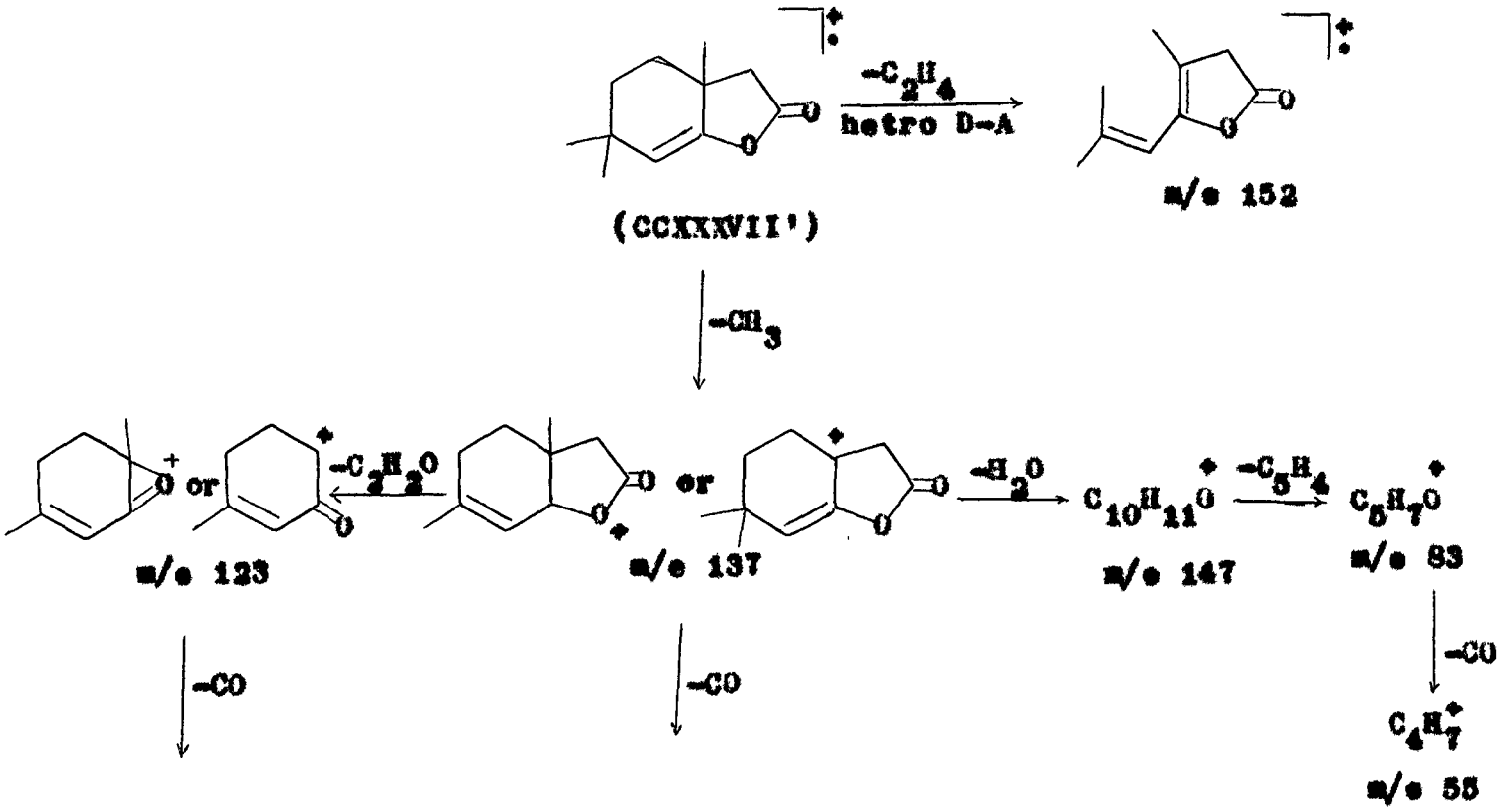


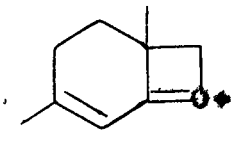
m/e 124, 110 and 96



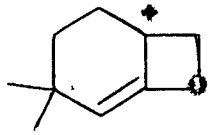
1,4,4-Trimethylcyclohexan-2-one acetic acid enol lactone (CCXXXVII)

Scheme - 7





or



$-C_4H_4O$

$C_5H_9^+$
m/e 69

$-C_2H_4$

$C_3H_5^+$
m/e 41

$-H_2$

$C_3H_3^+$
m/e 39

m/e 137

$-CO$

$C_8H_{13}^+$
m/e 109

$-C_2H_4$

$C_6H_9^+$
m/e 81

$-H_2$

$C_6H_7^+$
m/e 79

$-H_2$

$C_6H_5^+$
m/e 77

$C_7H_{11}^+$
m/e 95

$-C_2H_5$

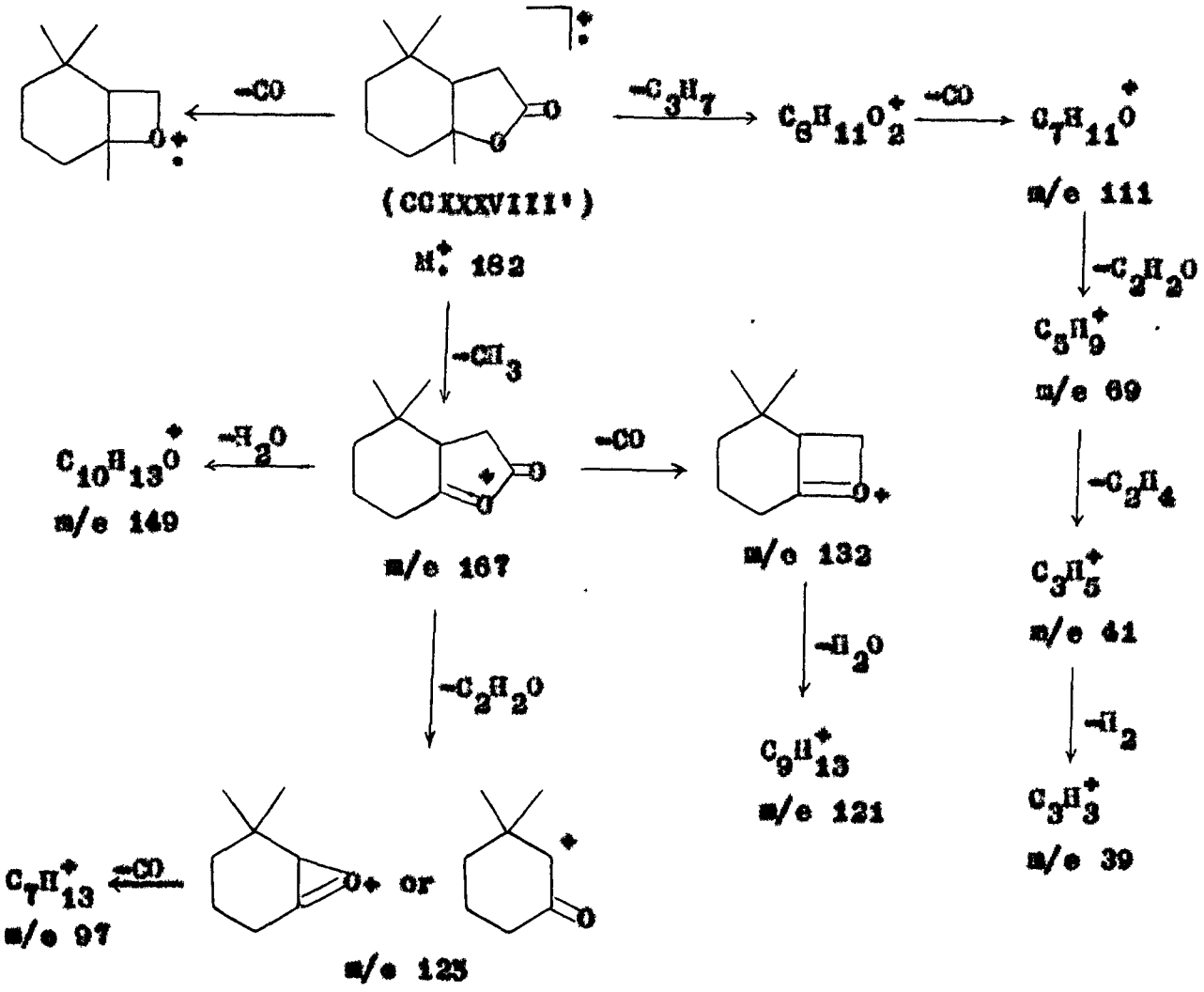
$C_5H_7^+$
m/e 67

$-H_2$

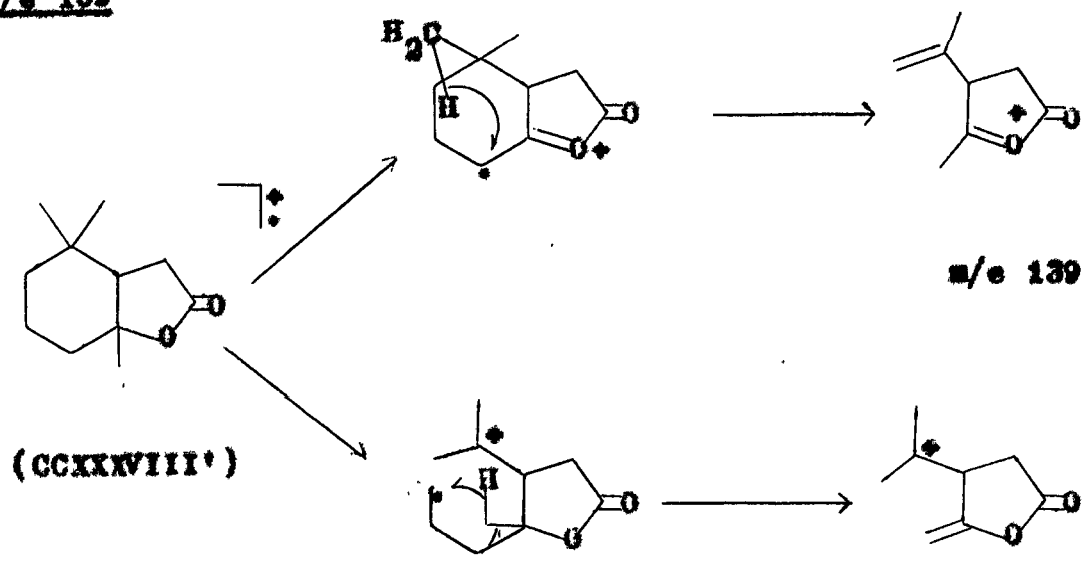
$C_5H_5^+$
m/e 65

Tetrahydronootinidiolide (CCXXXVIII)

Scheme - 8



m/e 139



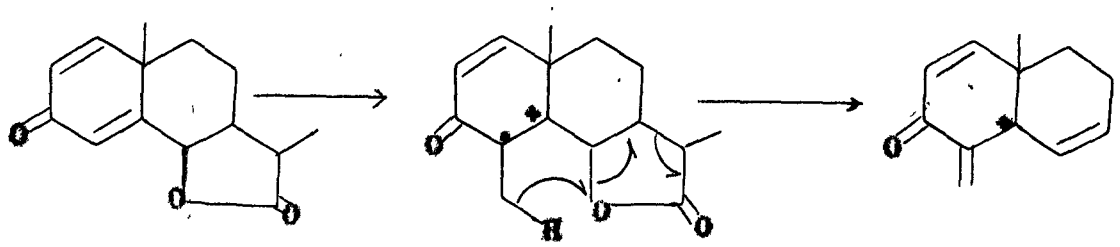
m/e 139

(CCXXXVIII')

m/e 139

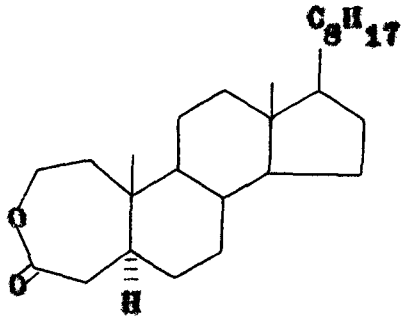
The most common feature, which has been observed in the mass spectra of santonins, such as (CCXXXIX), is associated with an abundant (M-73) ion[62,63]. The accurate mass measurement has shown it to be arising by the expulsion of γ-lactone ring[63-65] with one hydrogen atom. The process of dissociation was explained to begin by removal of a π-electron from C4-C5 double bond in (CCXXXIX) to give the molecular ion (CCXXXIX'). Hydrogen transfer from C4-methyl group in (CCXXXIX') followed by cleavage of the lactone ring, gives rise to the charged fragment (M-73)(C) (Scheme-9).

Scheme- 9

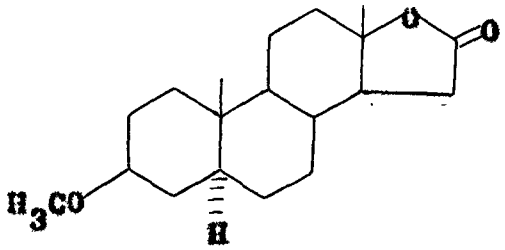


(CCXXXIX) (CCXXXIX') (c) M-73

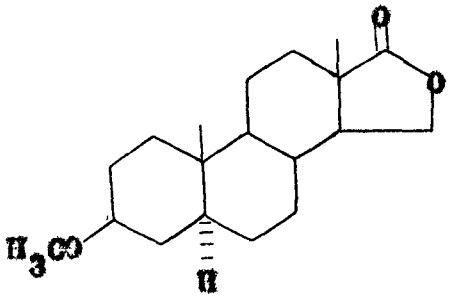
The mass spectra of steroidal lactones[66] have been used to examine the composition between a lactone-directed decomposition mode and the general fragmentation of a complex hydrocarbon frame work. It was found[66,67] that in the mass spectrum of 2-oxa-5α-cholestan-3-one (XIV), the usual fragmentation of steroid nucleus is prevailing. However, 3β-methoxy-17-oxa-5α-androstan-16-one (CCXL) exhibits a very intense M-CH_3 peak due to the possible formation of an oxonium species (c), while the isomeric 3β-methoxy-16-oxa-5α-androstan-17-one (CCXLI) shows methyl radical ejection only to a minor extent, since the elimination of the C18-methyl group is now relatively unfavourable[67]. The situation can be briefly stated that the influence of the lactone grouping is rather weak except for those cases in which it is located suitably to initiate a favoured breakdown process.



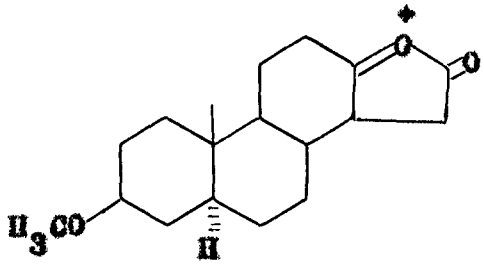
(XIV)



(CCXL)



(CCXLI)

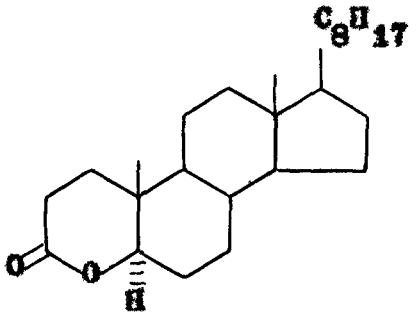


(a)

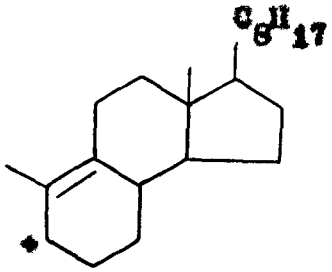
The mass spectral fragmentation patterns of steroidal ring A-lactones show that the prominent ion peaks arise due to the dissociative cleavage of the large hydrocarbon moiety.

2-Oxa-5α-cholestan-3-one (XIV)[66] gave significant ion peaks at m/e 388 ($M^{\cdot+}$), 373 ($M-CH_3$), 303 ($M-C_6H_{13}$), 275 ($M-C_8H_{17}$), 248 ($M-C_{10}H_{20}$), 234, 233 (lactone ring cleavage) and 219 (m/e 234-CH_3). The isomeric lactone, 4-oxa-5β-cholestan-3-one (IX) gave similar spectrum with ion peaks at m/e 388 ($M^{\cdot+}$), 373 ($M-CH_3$), 315 (lactone ring cleavage), 303, 287, 275, 248, 234, 233 and lower mass peaks.

The fragment ion at m/e 315 from the lactones (XIV) and (IX) is explained as arising due to the lactone ring cleavage and this species was formulated as (e).



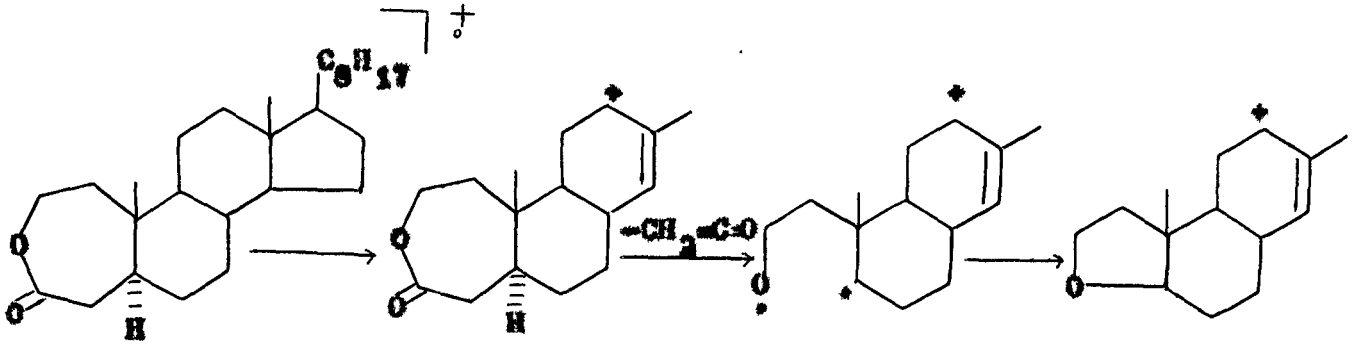
(IX)



(e)
m/e 315

In case of the ϵ -lactones, 3-oxa-A-homo-5α-cholestan-4-one (V) and its isomer, 4-oxa-A-homo-5α-cholestan-3-one (IV) similar behaviour has been observed. The lactone ring fragmentation leads to the formation of important fragments m/e 205 (f) and (g) and m/e 329 (h) which were shown to arise according to Schemes-10 and 11.

Scheme - 10

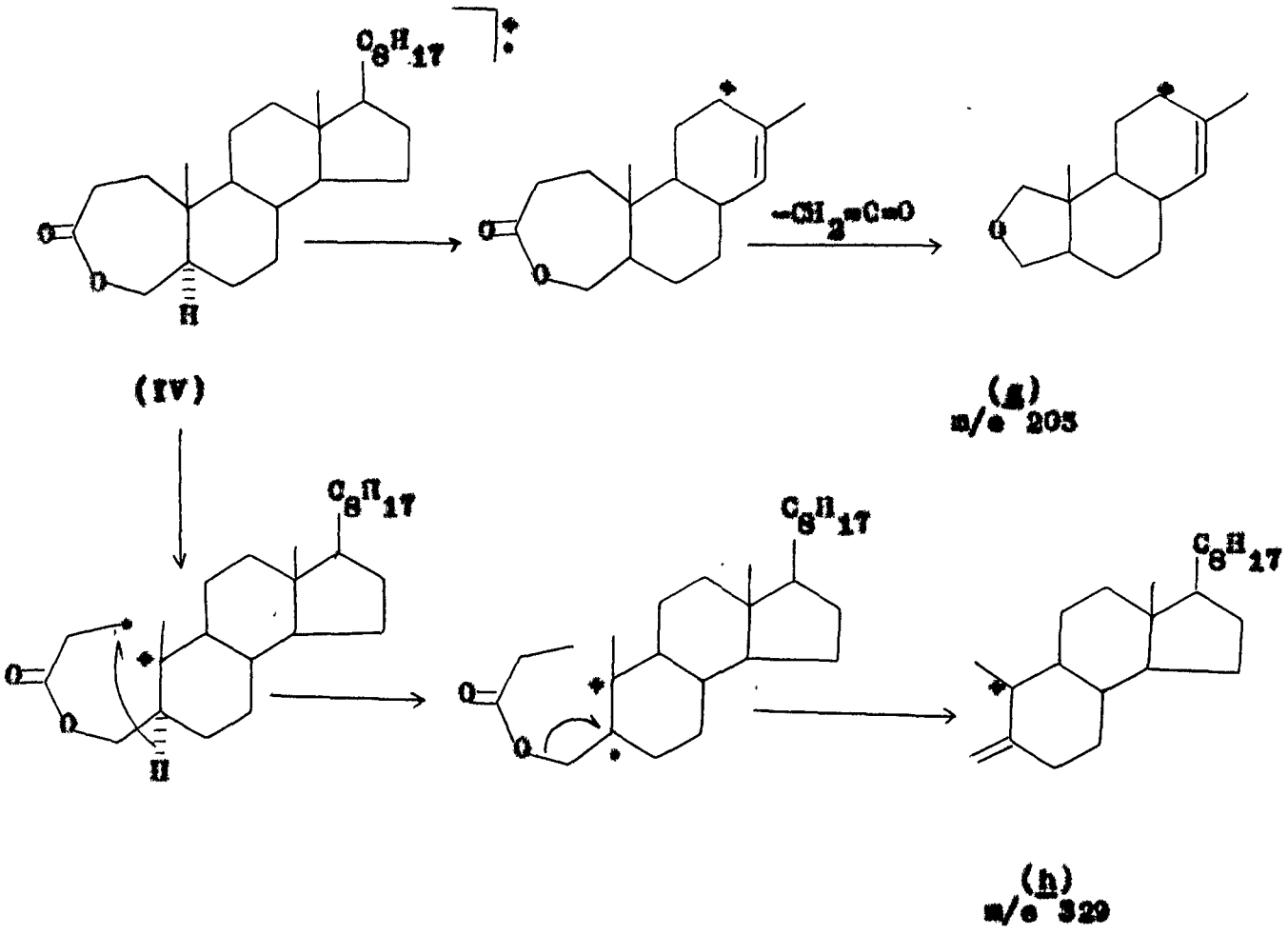


(V)

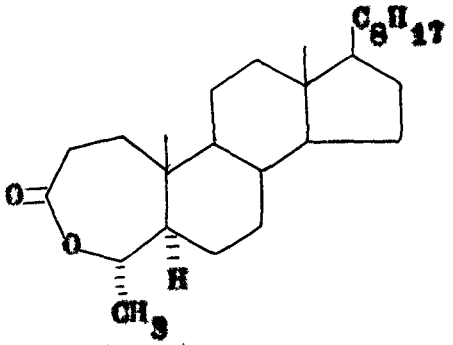
(f)
m/e 205

m/e 329 and 205

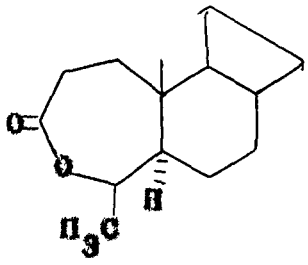
Scheme - 11



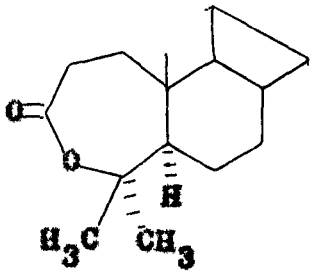
These spectral results have been supported by the corresponding spectra for different C4a-methyl substituted lactones, 4-oxa-A-homo-4aα-methyl-5α-cholestan-3-one (XXI), 4-oxa-A-homo-4aβ-methyl-5α-cholestan-3-one (XXII) and 4-oxa-A-homo-4a,4a-dimethyl-5α-cholestan-3-one (XXIII).



(XXI)

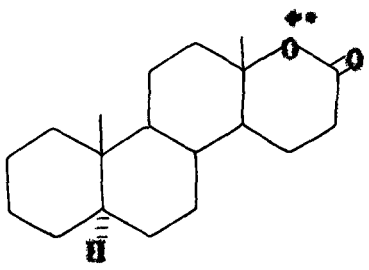


(XXII)

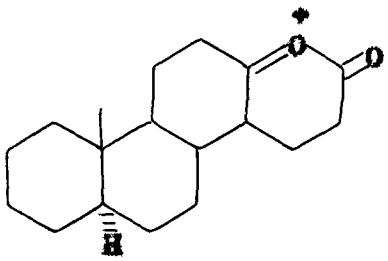


(XXIII)

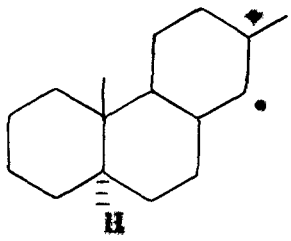
The main fragment ions in the mass spectrum of ring D δ-lactone, 17-oxa-D-homo-5α-androstan-16-one (CCXLII) were shown to arise by the loss of a methyl group and cleavage of the lactone ring to give fragments (i) and (j)[9].



(CCXLII')

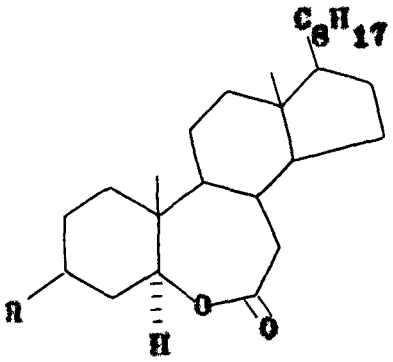


(i)

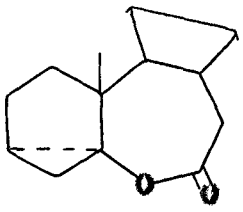


(j)

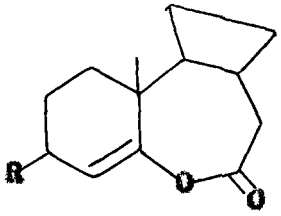
Recently, Ahmad et al.[68] have studied the mass spectra of steroidal ε-lactones, such as, 6-oxa-B-homo-5α-cholestan-7-one (XXXV), 3β-chloro-6-oxa-B-homo-5α-cholestan-7-one (CCXLIII), 3β-bromo-6-oxa-B-homo-5α-cholestan-7-one (CCXLIV), 6-oxa-B-homo-3α, 5-cyclo-5α-cholestan-7-one (CCXLV), 6-oxa-B-homocholest-4-en-7-one (CCXLVI) and 3β-acetoxy-6-oxa-B-homocholest-4-en-7-one (CCXLVII).



(XXXV) R, H
(CCXLIII) R, Cl
(CCXLIV) R, Br



(CCXLV)



(CCXLVI) R, H
(CCXLVII) R, OAc

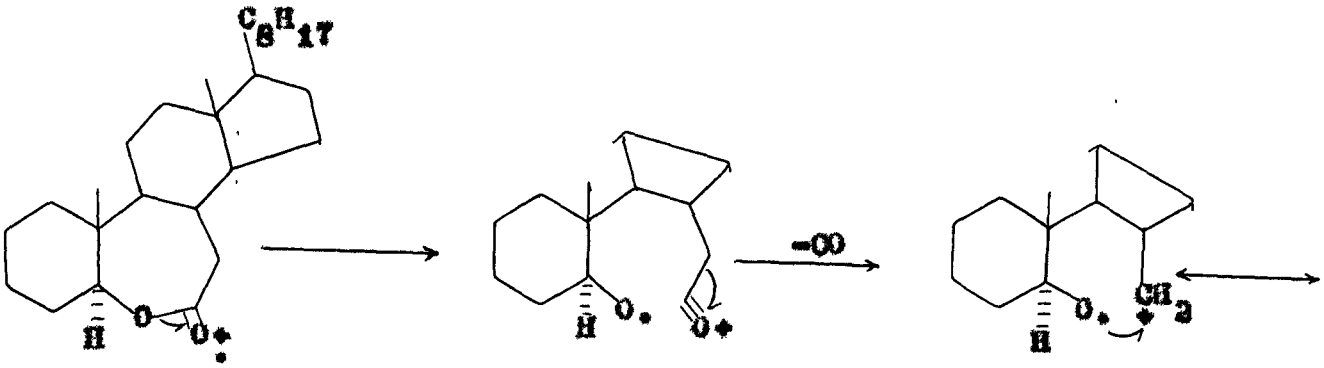
The mass spectrum of 6-oxa-8-homo-5α-cholestan-7-one (XXXV) gave molecular ion at m/e 402($C_{27}H_{46}O_2$) with other significant peaks at m/e 387 ($M-CH_3$), 384 ($M-H_2O$; $C_{27}H_{44}O$, ratio of M to $M-H_2O$ 1:1), 374 ($M-CO$; $C_{26}H_{46}O$), 360 ($M-CH_2=C=O$), 359 ($M-CO, CH_3$), 356 ($M-H_2O$, CO), 318 (base peak, $C_{22}H_{38}O$), 317 (m/e 318-H), 303 (m/e 318-CH_3), 289, 262 ($C_{19}H_{34}$), 247 ($C_{18}H_{31}$), 219, 179 ($C_{12}H_{19}O$) and lower mass peaks.

m/e 318 (base peak, $C_{22}H_{38}O$)

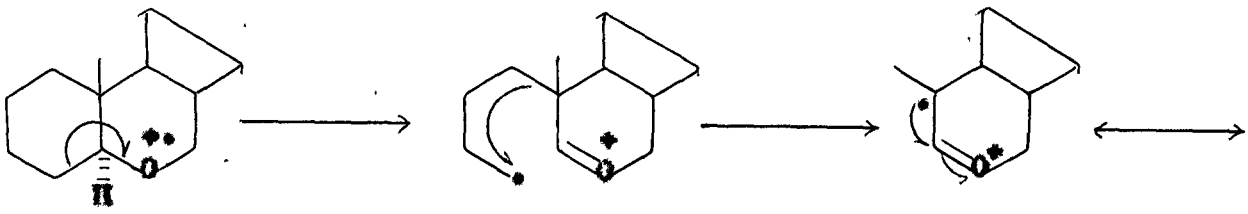
The fragment ion peak at m/e 318 constitutes the base peak of the spectrum. The accurate mass measurement showed the composition $C_{22}H_{38}O$. This fragment ion apparently results by the loss of CO and ring A. This indeed seems to be the case since this ion peak is present in the mass spectra of the lactones (CCXLIII), (CCXLIV) and (CCXLV).

The formation of the fragment ion m/e 318 from (XXV) has been suggested to occur according to Scheme - 12.

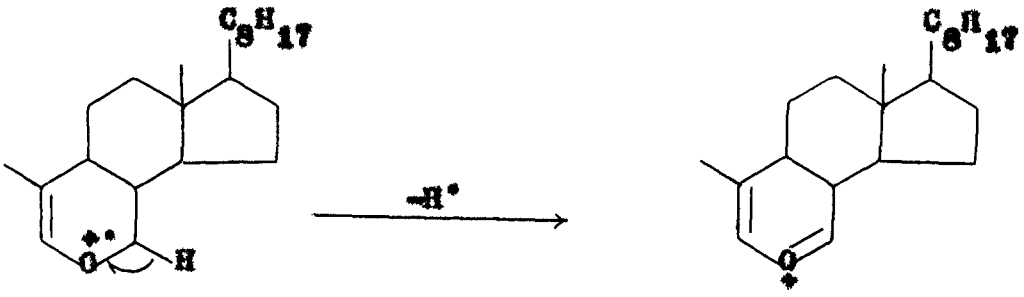
Scheme - 12



(XXXV)



(k)
m/e 374 ($C_{26}H_{46}O$)



(l)
m/e 318 ($C_{22}H_{38}O$)

(m)
m/e 317 ($C_{22}H_{37}O$)

The mass spectrum of the chloro-lactone (CCXLIII) gave molecular ion peaks at m/e 436/438 (3:1) followed by other significant peaks at m/e 421/423 (M-CH_3), 418/420 (M-H_2O), 408/410 (M-CO), 401 (M-Cl), 400 (M-HCl), 385 (m/e 400-CH_3), 372 (m/e 400-CO), 318 (base peak, $C_{22}H_{38}O$), 317, 262 ($C_{19}H_{34}$), 247 ($C_{18}H_{31}$), 178, 109, 108 and lower mass peaks.

The mass spectrum of the bromo-lactone (CCXLIV) gave molecular ion peaks at m/e 480/482 (1:1) and other comparable peaks at m/e 465/467 (M-CH_3), 462/464 (M-H_2O), 452/454 (M-CO), 401 (M-Br), 400 (M-HBr), 385 (m/e 400-CH_3), 383 (m/e 401-H_2O), 373 (m/e 401-CO), 372 (m/e 400-CO), 318, 262, 247, 109, 108 and lower mass peaks. The spectra of both (CCXLIII) and (CCXLIV) can be easily correlated with that of (XXXV).

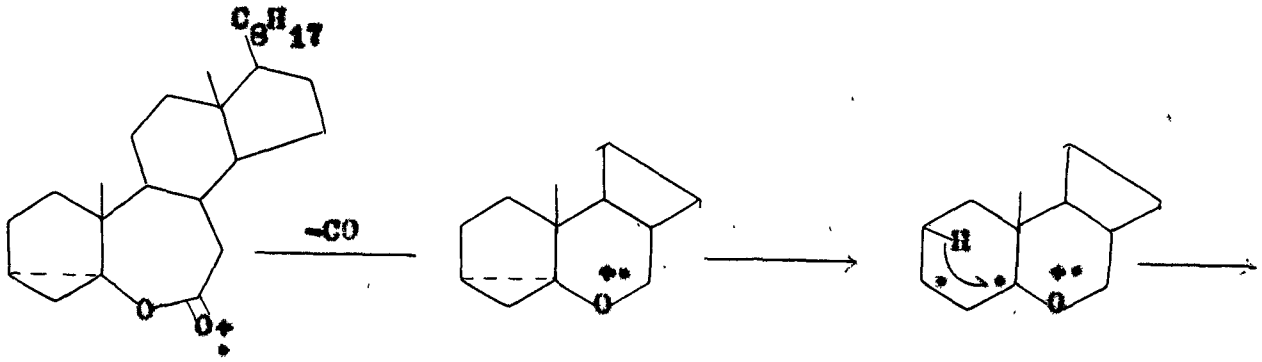
The mass spectrum of 3$\prec$,5$\prec$-cyclo-ϵ-lactone (CCXLV) gave molecular ion peak at m/e 400 ($C_{27}H_{44}O_2$) followed by other significant peaks at m/e 386 (M-CH_3), 382 (M-H_2O; ratio of M to M-H_2O, 2.5:1), 372 (M-28), 359 (M-41), 357 (m/e 372-CH_3), 354 (m/e 372-H_2O, M-46), 344 (m/e 372-28), 339 (m/e 354-CH_3), 331 (m/e 359-CO), 330, 329, 318 ($C_{22}H_{38}O$), 317, 302 (m/e 318-CH_3), 289, 287, 247, 245, 111, 110 and lower mass peaks.

m/e 318 ($C_{22}H_{38}O$)

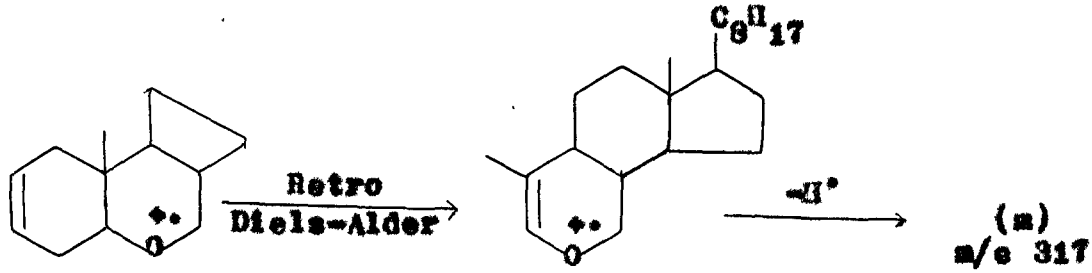
As in the spectra of the preceding lactones (XXXV)

(CCXLIII) and (CCXLIV), the spectrum of (CCXLV) also exhibited a prominent peak at m/e 318 ($C_{22}H_{38}O$). Its genesis, however, could not be shown according to Scheme-12. As an alternative, Scheme-13 has been proposed for the formation of this important ion from (CCXLV).

Scheme - 13



(CCXLV)

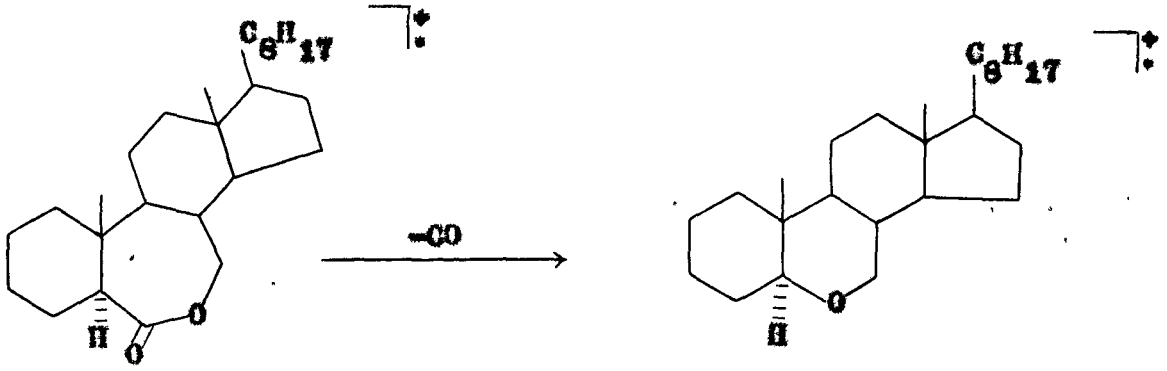


(1)
m/e 318

(m)
m/e 317

Though they found it tempting to suggest that the fragment ion m/e 318 is of real diagnostic value in the characterization of

ϵ-lactones of the type (XXXV), (CCXLIII), (CCXLIV) and (CCXLV), but at the best it should be considered of a limited value. According to them, the fragment ion m/e 374 (k from XXXV) from which the ion m/e 318 has been suggested to arise can also be derived from the isomeric 7-oxa compound (CCXLVIII). Therefore, the mass spectral results should be considered in conjunction with other methods of characterisation.



(CCXLVIII') (k) m/e 374

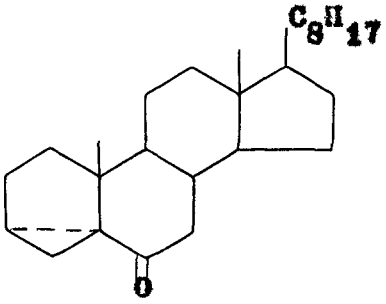
In order to test the validity of the mechanism proposed for the formation of the ion m/e 318, they examined the mass spectra of (CCXLVI) and its 3β-acetate analogue (CCXLVII). This mechanism finds support from the fact that the mass spectra of (CCXLVI) and (CCXLVII) do not show the presence of the fragment ion peak at m/e 318 as there is no rationale for the loss of ring A either from (CCXLVI) or (CCXLVII) which would involve the cleavage of C_4-C_5 double bond.

DISCUSSION

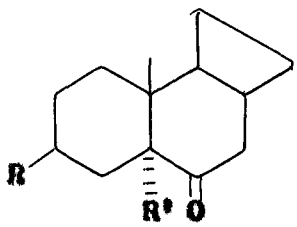
Baeyer-Villiger oxidation of steroidal ketones

Discussion

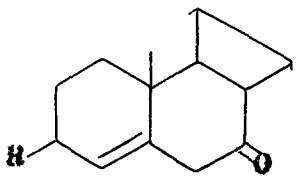
In the preceding years, a number of communications from this laboratory described the results of Baeyer-Villiger oxidation of steroidal ketones, both saturated as well as α,β-unsaturated ones. These reactions provided a variety of interesting rearranged products and their formation depended to a large extent on the peracid employed, the catalyst used and the reaction period. The substrates on which previous studies centred were 3α,5-cyclo-5α-cholestan-6-one (CCX), its 3β-halodorivatives (CCXLIX - CCLI)[69], 5-bromo-5α-cholestan-6-one (XXXVII), its 3β-acetoxy analogue (XXXVIII)[14], cholest-5-en-7-one (XCI), its 3β-acetoxy analogue (LXXXVI)[34], cholesta-3,5-dien-7-one (CCXXX)[70], cholesta-2,4-dien-6-one (CV)[36], 6β-bromocholest-4-en-3-one (LXXXIII)[33], 4α-acetoxy-cholest-5-en-3-one (XXIV)[10], methyl 5-keto-5,6-secocholestan-6-oate (CVIII), methyl 5-keto-5,6-secocholest-3-en-6-oate (CX) and methyl 5-keto-4,5-secocholestan-4-oate (CIX)[37].



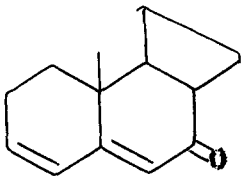
(CCX)



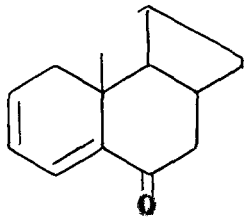
(CCXLIX) R, Cl; R',H
(CCL) R, Br; R',H
(CCLI) R, I ; R',H
(XXXVII) R, H ; R',Br
(XXXVIII) R, OAc; R',Br



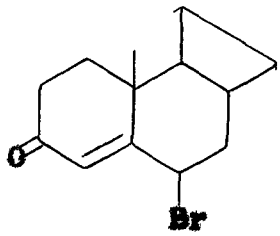
(XCI) R, H
(LXXXVI) R, OAc



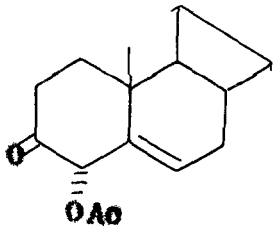
(CCXXX)



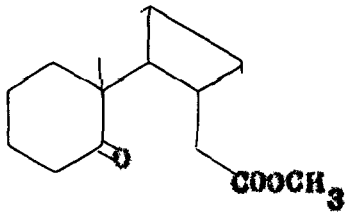
(CV)



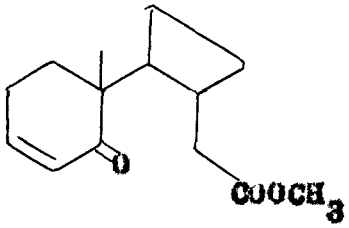
(LXXXIII)



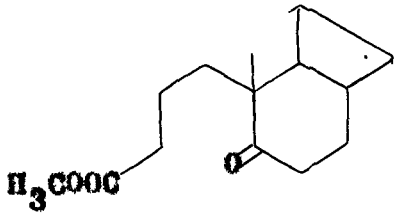
(CXXIV)



(CVIII)



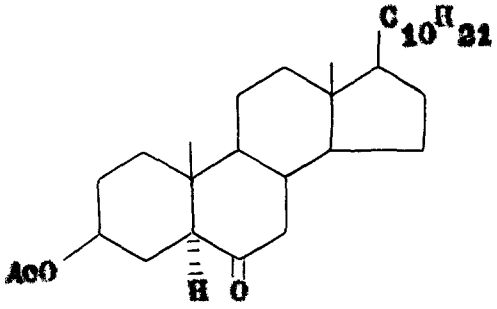
(CX)



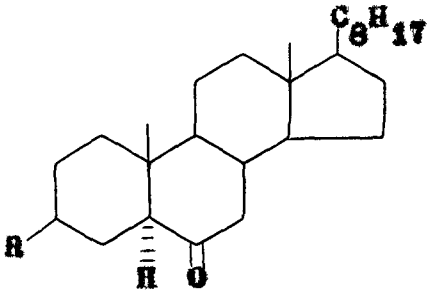
(CIX)

In continuation of our interest in this area, we attempted to explore the Baeyer-Villiger oxidation of hitherto unexplored ketones derived from β-sitosterol, and our first step in this direction was the perbenzoic acid oxidation of 6-oxo-5α- β-sitostanyl acetate (CCLII). In the light of the results obtained with (CCLII) we repeated peracid oxidation of several other steroidal 6-ones, such as (XXXIV), (CCLIII), (XXXIII), (CCXLIX), (CCL) and (CCX). In addition to this, we also accomplished the

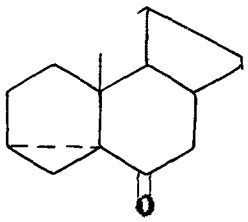
Baeyer-Villiger oxidation of 5α-cholestane-3,6-dione (CCXV), 3β-acetoxycholest-4-en-6-one (CCLIV) and cholest-4-ene-3,6-dione (CCXXI), the results of which will be described later.



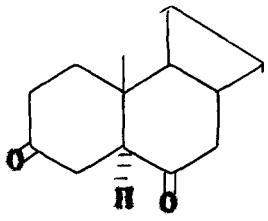
(CCLII)



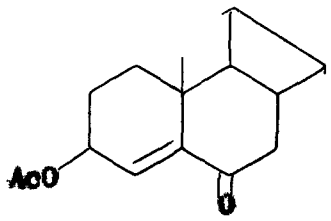
(XXXIV) R, OAc
(CCLIII) R, OH
(XXXIII) R, H
(CCXLIX) R, Cl
(CCL) R, Br



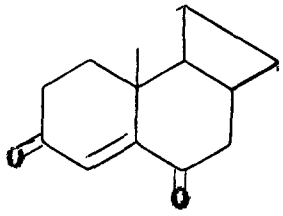
(CCX)



(CCXV)



(CCLIV)

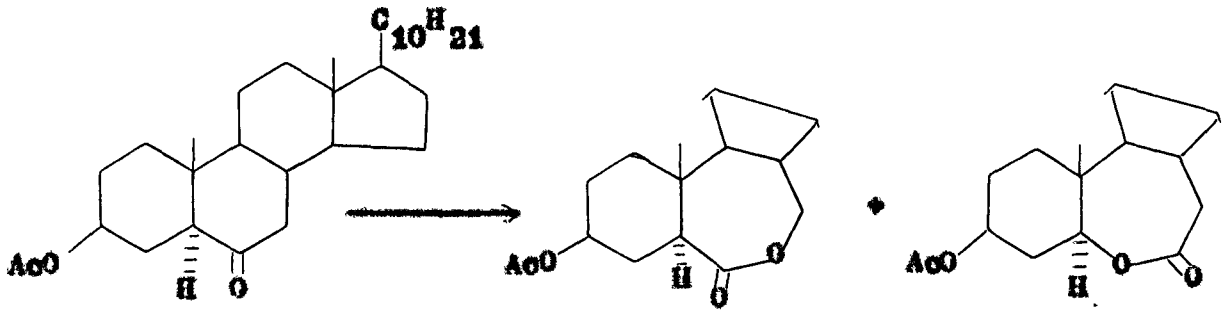


(CCXXI)

A. Saturated ketones

The Baeyer-Villiger oxidation of 6-oxo-5α-β-sitostanyl acetate (CCLII)

6-Oxo-5α-β-sitostanyl acetate (CCLII) was submitted to Baeyer-Villiger oxidation using perbenzoic acid (1.1 mole equivalent) as oxidant and p-toluenesulphonic acid monohydrate as catalyst. Usual work up of the reaction mixture and column chromatography over silica gel provided two compounds, m.pts. 130-31° and 163-64°.



(CCLII) (CCLV) (CCLVI)

Characterization of the compound, m.p. 130-31° as 7-oxa-B-homo-6-oxo-5α-β-sitostanyl acetate (CCLV)

The compound, m.p. 130-31° analysed for $C_{31}H_{52}O_4$ and this composition was supported by its mass spectrum showing molecular ion peak at m/e 488 ($C_{31}H_{52}O_4$). Thus only one oxygen atom was indicated to have been added during the course of the reaction. Its i.r. spectrum showed bands at 1740s, 1715s, 1250s, 1210m

and 1040m cm^{-1}. The bands at 1740 and 1715 are assignable to the acetate and ϵ -lactone carbonyls, respectively, and those at 1250, 1210 and 1040 for acetate and C-O stretching. On the basis of these i.r. values and the previous observations that 6-oxo-steroids on Baeyer-Villiger oxidation lead exclusively to the 6-oxasteroid by superior migratory aptitude of a more substituted tertiary C5 relative to secondary C7, we anticipated this compound to be the 6-oxasteroid. However, its n.m.r. spectrum revealed an altogether different picture. A broad signal at δ 4.66 ($\nu\frac{1}{2}$ 14 Hz) integrating for one proton was easily ascribable to C3-axial proton (α). There was a broadened singlet at δ 4.08(1H) and a doublet with J value of 3.5 Hz at 3.98 (1H). This downfield signal for two protons was a clear indication of a methylene group linked to oxygen which could only be the C7a-methylene group i.e. $-CH_2-O-\overset{O}{\overset{\|}{C}}-$ grouping is present. In view of the fact that equatorial protons resonate at a lower field compared to axial protons in the chair form of cyclohexane system, these latter signals were thought to be those of C7a pseudo equatorial (β) and pseudo axial (α) protons, respectively. But their appearance as a broadened singlet and doublet, respectively, was difficult to comprehend. For an understanding, the Dreiding model of the lactone (CCLV) was examined which showed the dihedral angle between C8 axial (β) and C7a-β(pseudo equatorial) protons to be almost 90^0 which may account for the absence of its (C7a-βH) splitting with C8-βH.

On the other hand, C7a-α proton is split by C8-βH into a doublet with J value of 3.5 Hz. If it was a genuine axial proton, J value would have been much higher. A double doublet (J = 11 Hz and 5 Hz) centred at δ 2.9 integrating for 1 proton can be assigned to C5-axial (α) proton which is coupled with C4-axial (β) proton to a magnitude of 11 Hz and with C4-equatorial (α) proton to the extent of 5 Hz. The acetate methyl protons appeared as a sharp singlet at δ 2.01. Other signals were observed at δ 0.9, 0.8 and 0.7 (6 methyl groups). On the basis of the foregoing discussion the compound, m.p. 130-31° has been characterized as 7-oxa-B-homo-6-oxo-5α-β-sitostanyl acetate (CCLV).

Characterization of the compound, m.p. 163-64° as 6-oxa-B-homo-7-oxo-5α-β-sitostanyl acetate (CCLVI)

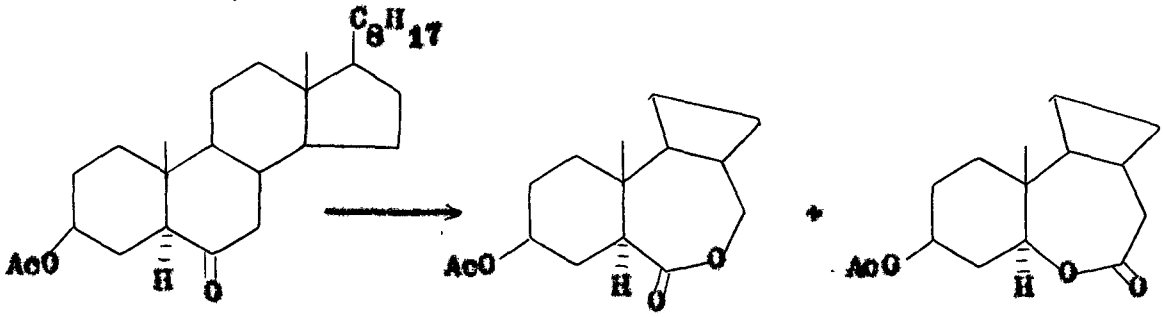
This compound was shown to be an isomer of the 7-oxa compound (CCLV) by its analysis ($C_{31}H_{52}O_4$) and the molecular ion peak (m/e 488) in its mass spectrum. Its i.r. spectrum showed bands at 1740s (acetate carbonyl), 1720s (ϵ-lactone), 1240s (acetate) and 1040s cm^{-1} (C-O). That this is the 6-oxa compound, which was expected to be formed exclusively, was proved by its n.m.r. spectrum wherein a broad multiplet at δ 4.75 integrating for one proton could be assigned to C3-axial (α) proton with a half band width of 14 Hz. A double doublet (J = 11 Hz and 5 Hz) centred at δ 4.29 integrating for one proton indicated the presence of a methine proton adjacent to oxygen which can obviously be

assigned to C3-axial (α) proton which is coupled with C4-axial (β) and C4-equatorial (α) protons with J values of 11 Hz and 5 Hz, respectively, i.e. CH_2-C(H)-O-C(=O)- group is present. A pattern, similar to that seen in (CCLV) was observed for C7a protons in this case also. A broadened singlet for C7a-βH (pseudo equatorial) was observed at δ 2.5 and a doublet for C7a-αH (pseudo axial) at 2.43 (J 3.5 Hz). The Dreiding model of this lactone also revealed an identical relationship between C3-βH and C7a-H_2 as noted in (CCLV). A sharp singlet for 3 protons was observed at δ 2.03 which was due to acetate methyl protons. Other signals were seen at δ 0.9, 0.8 and 0.7 (6 methyl groups).

This reaction showed results different from the earlier observations[13,69] where the Baeyer-Villiger oxidation of 6-keto-steroids was reported to be a highly stereospecific process providing exclusively the 6-oxa steroid by greater migratory tendency of a more substituted carbon (C5). It was noted that in the present case, the 7-oxa compound was obtained in almost equal yield relative to 6-oxa compound. This observation was significant in the sense that the previous 'stereospecific' oxidation of steroidal 6-ones could not be accepted without doubts. Thus this observation prompted us to restudy the peracid oxidations of those 6-keto-steroids which in the past were claimed to provide only the 6-oxasteroids.

The Baeyer-Villiger Oxidation of 3β-acetoxy-5α-cholestan-6-one (XXXIV)

The ketone (XXXIV) was oxidised with perbenzoic acid (1.1 mole equivalent) in the usual manner. Two products, m.pts. 181° and 174° were obtained in about equal amounts from this reaction after usual work up and column chromatography.



(XXXIV) (CCLVII) (XXXVI)

Characterization of the compound, m.p. 181° as 3β-acetoxy-7-oxa-B-homo-5α-cholestan-6-one (CCLVII)

The compound, m.p. 181° analysed for $C_{29}H_{48}O_4$ and this composition was substantiated by its mass spectrum (M^+ 460). Thus addition of only one oxygen atom to the parent ketone was shown. This compound was also indicated to be the 7-oxasteroid by its spectral analogy to the 7-oxa compound (CCLV) isolated from the previous reaction. Its i.r. spectrum showed bands at 1740s ($CH_3\underline{COO}$-), 1715s (ε -lactone), 1250s (acetate), 1205 and 1035 cm^{-1} (C-O). In the n.m.r. spectrum, a broad signal for 1 proton at δ 4.66 ($W_{\frac{1}{2}}$ 14 Hz) was assigned to C3-proton (α).

A broad singlet for 1 proton was observed at δ 4.1 (C7a-βH) and a doublet for 1 proton was seen at δ 4.0 (C7a-αH, J 3.5 Hz). A double doublet for 1 proton was noted at δ 2.92 (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz). The acetate methyl protons appeared as a sharp singlet at δ 2.03. The methyl group signals were observed at δ 0.9, 0.8 and 0.7 (for 5 methyl groups).

Characterization of the compound, m.p. 174° as 3β-acetoxy-6-oxa-3-homo-5α-cholestan-7-one (XXXVI)

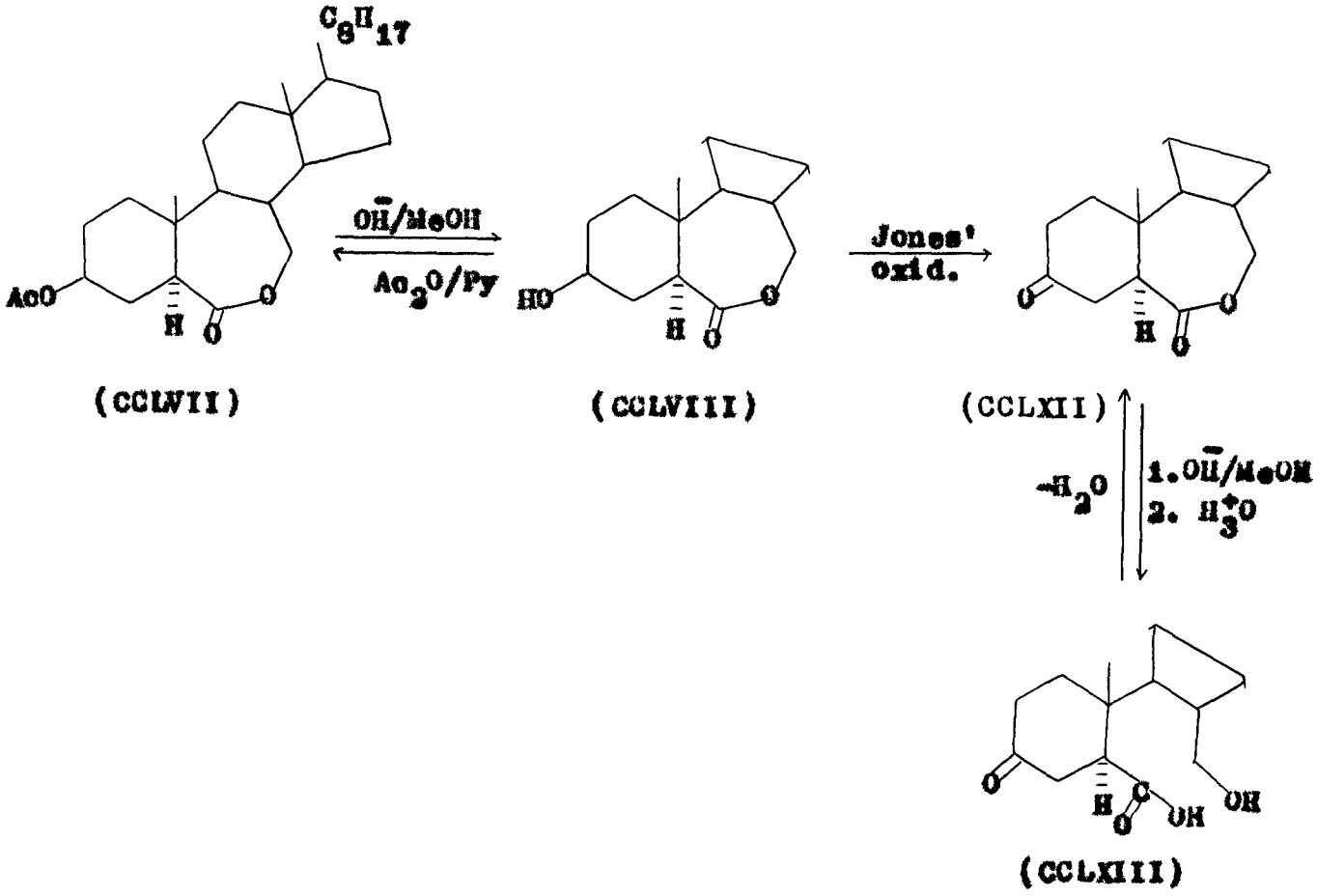
The compound, m.p. 174° analysed for $C_{29}H_{46}O_4$, and this composition was supported by its mass spectrum (M^+ 460). This compound was shown to be the 6-oxa isomer on the basis of its spectral similarity to (CCLVI). Its i.r. spectrum showed absorptions at 1740s (CH_3-COO), 1718s (ϵ-lactone), 1245s (acetate) and 1035s cm^{-1} (C-O). The n.m.r. spectrum gave a broad signal at δ4.75 for 1 proton ascribable to C3-αH ($W_{\frac{1}{2}}$ 14 Hz). A double doublet integrating for 1 proton appeared at δ 4.29 (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 3.5 Hz). A broad singlet integrating for 1 proton was seen at δ 2.5 (C7a-βH) and a doublet for 1 proton was observed at δ 2.42 (C7a-αH, J 3.5 Hz). Acetate methyl group appeared as a sharp singlet at δ 2.0. Other signals were seen at δ 0.9, 0.8 and 0.7 (5 methyl groups).

It is worth pointing out here that m.p. of the lactone (XXXVI) as reported by Fonken and Miles[13] is 162-63° whereas in

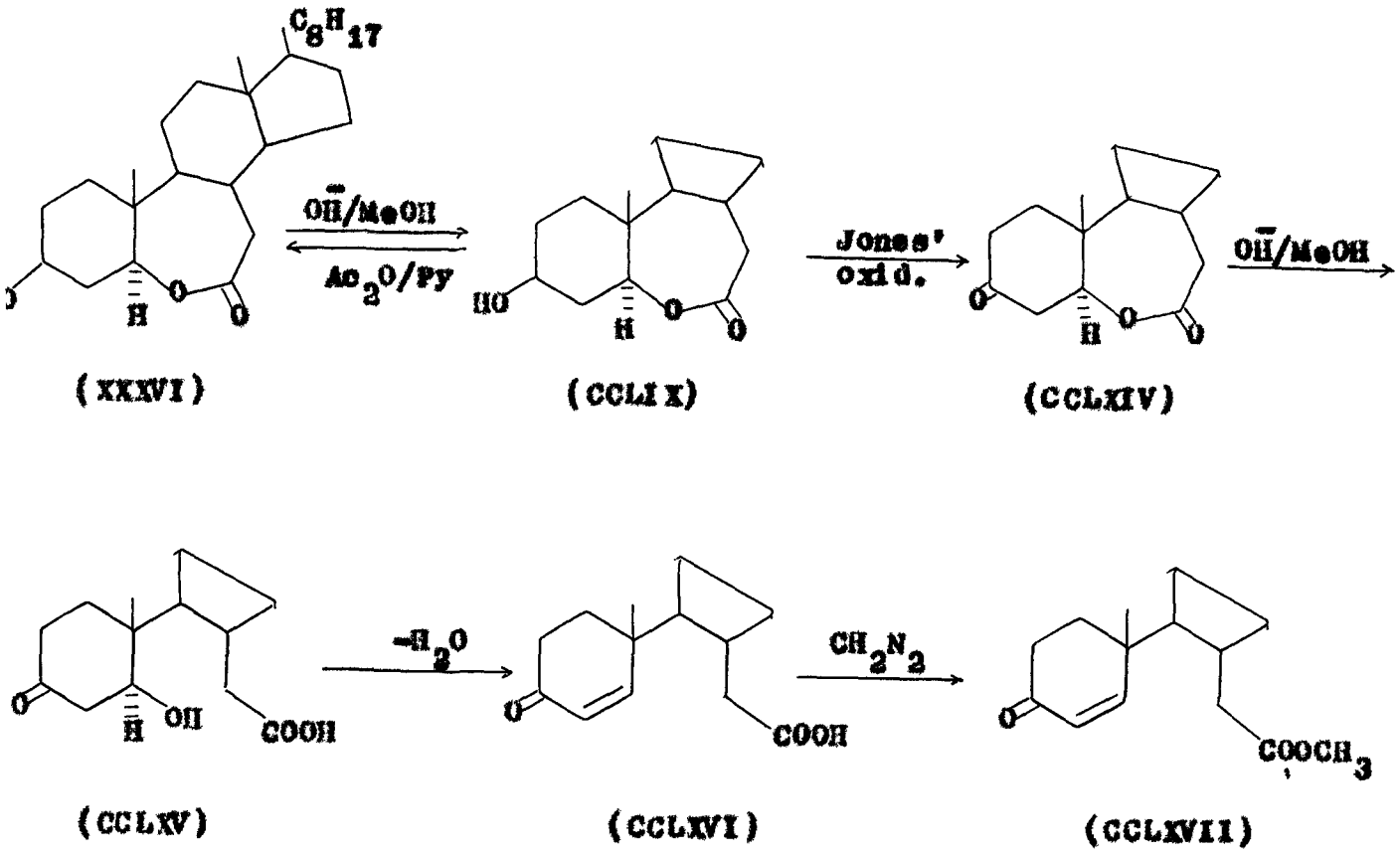
our hands it melted at 174°. From this difference in melting point it was suspected that the 'product' reported by Fonken and Miles to be (XXXVI) might actually have been a mixture of (XXXVI) and its isomer (CCLVII). An admixture of (XXXVI) and (CCLVII) in the ratio of 1:1 melted at 162-63° indicating that this indeed was the case.

In order to obtain chemical support in favour of the isomeric lactones (CCLVII) and (XXXVI), they were subjected to a sequence of reactions as outlined in the flowsheet given below. Rationalization of these observations throw light on the structure of the lactones (CCLVII) and (XXXVI).

Flow sheet



Flow sheet (Contd.)



(XXXVI) (CCLIX) (CCLXIV)

(CCLXV) (CCLXVI) (CCLXVII)

Base hydrolysis of (CCLVII): 3 -hydroxy-7-oxa-B-homo-5 - cholestan-6-one (CCLVIII)

Base hydrolysis of the acetate function in (CCLVII) gave a compound, m.p. 124° which analysed for $C_{27}H_{46}O_3$ and was characterized as the hydroxy lactone (CCLVIII) on the basis of its i.r. spectrum which exhibited bands at 3430br (OH), 1715s (ϵ-lactone), 1195 and 1060 cm^{-1}(C-O). The hydroxy lactone (CCLVIII) on pyridine-acetic anhydride treatment gave back the acetate lactone (CCLVII).

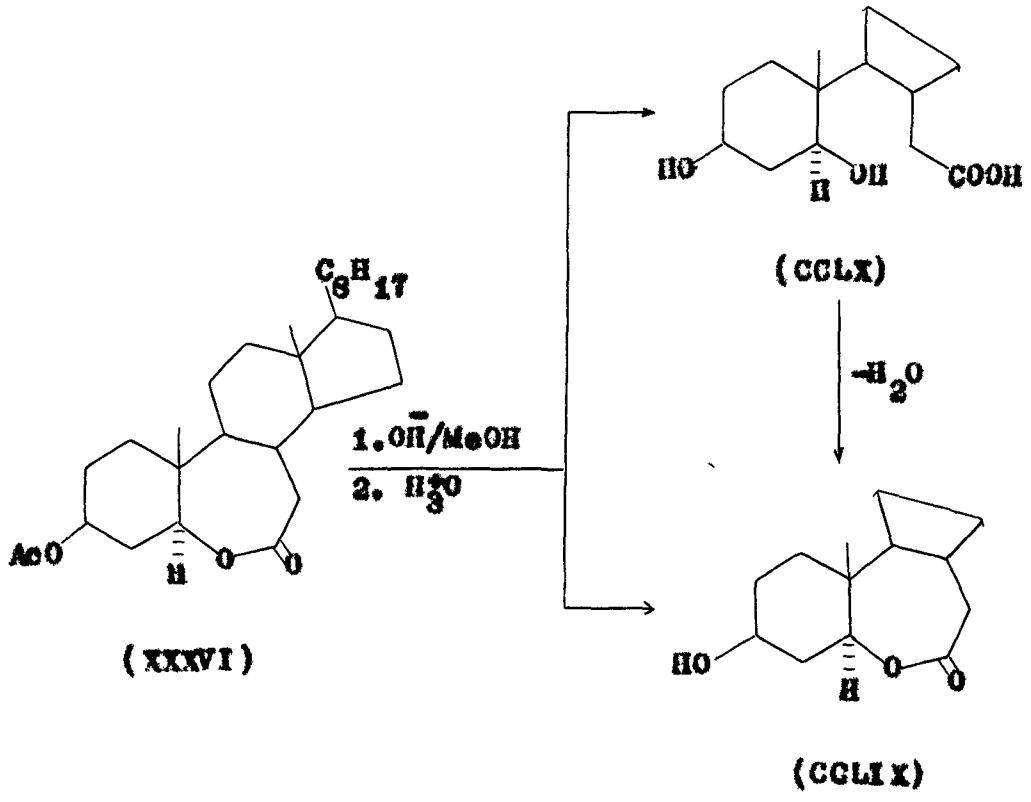
Base hydrolysis of (XXXVI): 3β-hydroxy-6-oxa-B-homo-5α-cholestan-7-one (CCLIX)

The acetate function in the lactone (XXXVI) was hydrolysed to give a compound, m.p. 202° which analysed for $C_{27}H_{46}O_3$. Its i.r. spectrum showed bands at 3300br (OH), 1718s (∈-lactone), 1225 and 1025 cm^{-1} (C-O). On the basis of the foregoing spectral values and its ability to regenerate (XXXVI) on acetylation, the compound, m.p. 202° is regarded as (CCLIX).

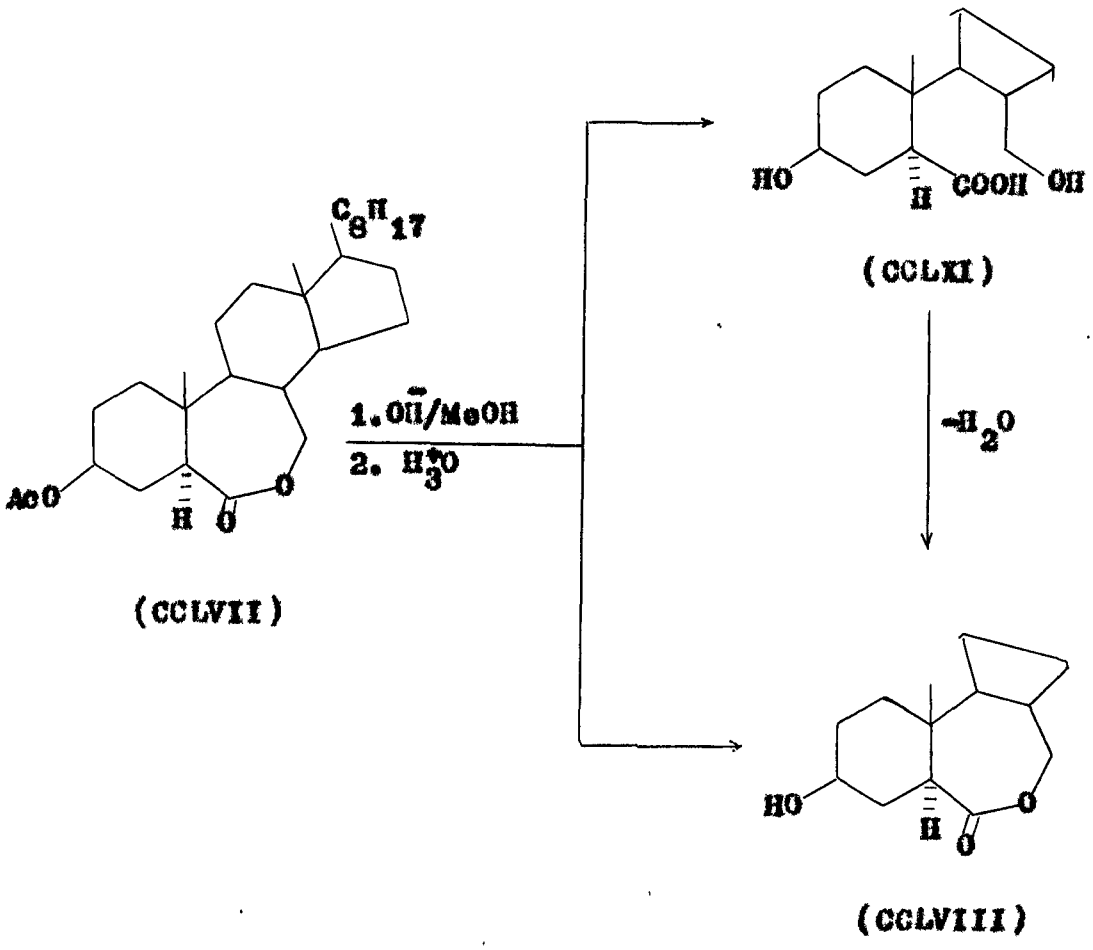
The m.p. of the hydroxy lactone (CCLIX), reported by Fonken and Miles[13] is 139-41° whereas we found it to be 202°. As the initial product of oxidation reported by them seems to have been a mixture of (XXXVI) and (CCLVII), its acetate hydrolysis would only have given rise to another mixture of isomeric hydroxy lactones (CCLVIII) and (CCLIX). This was proved by the m.p. of an admixture of (CCLVIII) and (CCLIX) which coincided with reported m.p. 139-41°of (CCLIX).

Note: It is pertinent to mention that according to Fonken and Miles[13] the alkaline hydrolysis of (XXXVI) gave the hydroxy lactone (CCLIX) and the dihydroxy secoacid (CCLX) depending upon the care taken in making the solution acidic after diluting with water. We observed that quick work up of even neutral solution provided a mixture, in about equal amount, of the hydroxy lactone (CCLIX) and the secoacid (CCLX). Efforts were made to separate them by column chromatography over silica gel but the secoacid (CCLX)

relactonized during the passage through silica gel as elution afforded only the hydroxy lactone (CCLIX). Further, when the mixture of the hydroxy lactone (CCLIX) and the secoacid (CCLX) was allowed to stand in a solvent after work up at room temperature, the latter started relactonising and after sometime the secoacid (CCLX) was completely converted into the hydroxy lactone (CCLIX). The tendency for relactonisation of hydroxy acids, such as (CCLX) is well known and needs no special comment.



Similar was the case with the isomeric 7-oxa lactone (CCLVII) from which we could not isolate the secoacid (CCLXI) upon hydrolysis.



(CCLVII)

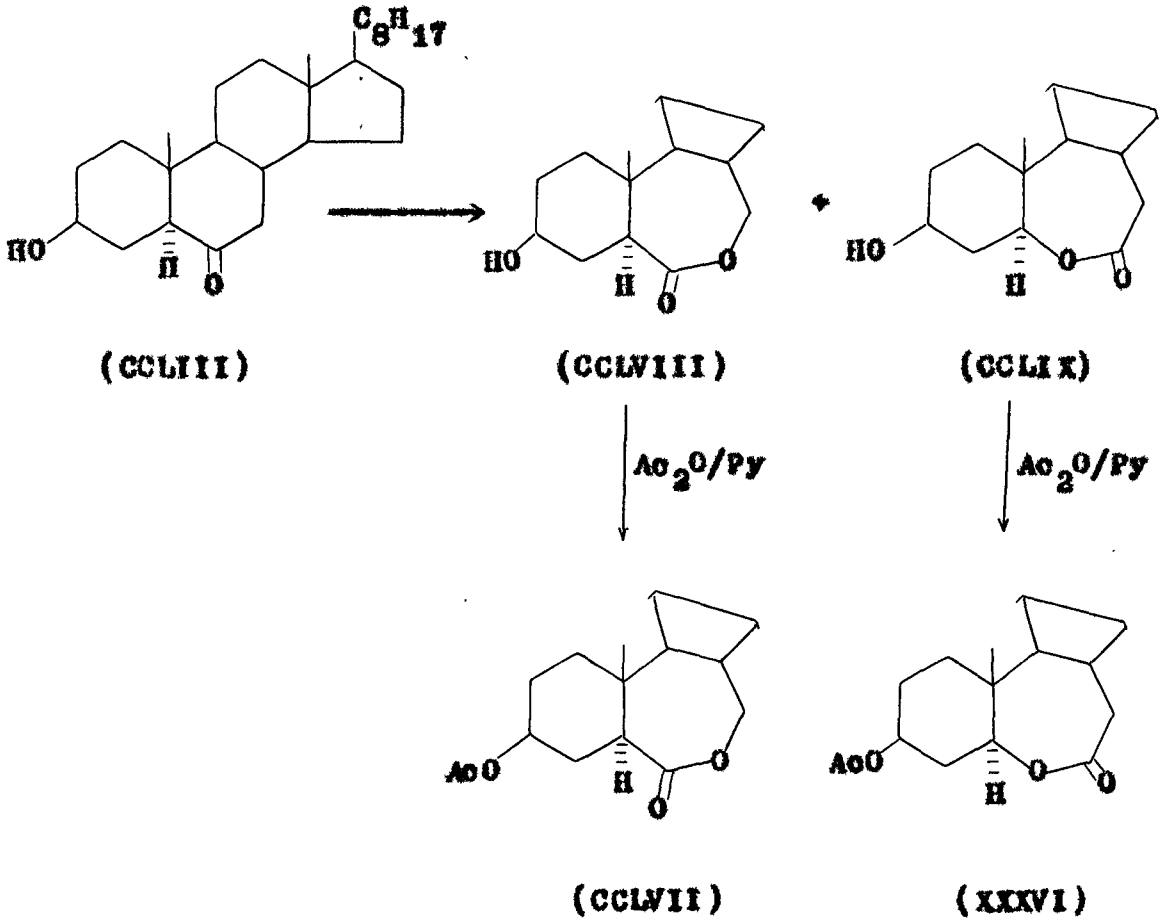
(CCLXI)

(CCLVIII)

The Baeyer-Villiger oxidation of 3β-hydroxy-5-α-cholestan-6-one (CCLIII)

In order to substantiate the structure of isomeric hydroxy-lactones (CCLVIII) and (CCLIX), the hydroxy ketone(CCLIII) was oxidised in the usual manner which gave two products, m.pts.

124° and 202° in almost equal amounts. These products were found to be identical with the hydroxy lactones (CCLVIII) and (CCLIX), obtained on hydrolysis of acetate group in the lactones (CCLVII) and (XXXVI), respectively. Treatment of the compound, m.p. 124° with pyridine-acetic anhydride gave the lactone (CCLVII) while the compound, m.p. 202° on similar treatment afforded the lactone (XXXVI).



(CCLIII) (CCLVIII) (CCLIX)

(CCLVII) (XXXVI)

7-Oxa-B-homo-5∝-cholestane-3,6-dione (CCLXII)

The hydroxy lactone (CCLVIII) on Jones' oxidation[71] gave a compound, m.p. 195° which analysed for $C_{27}H_{44}O_3$ and was characterised as the oxolactone (CCLXII) on the basis of its i.r. spectrum which gave bands at 1725s (∈-lactone), 1720s (C=O), 1185 and 1085 cm^{-1}(C-O).

Attempted hydrolysis of the oxolactone (CCLXII)

The oxolactone (CCLXII) was subjected to usual alkaline hydrolysis but we failed to isolate the secoacid (CCLXIII). It is reasonable to believe that (CCLXIII) is formed but readily undergoes relactonisation to furnish (CCLXII). This assumption is supported by the observation that the immediate t.l.c. of the hydrolysate from (CCLXII) shows the presence of two components (one of which is CCLXII). However, when the mixture is allowed to stand at room temperature for sometime (3-4 hours) or subjected to chromatographic separation only (CCLXII) is obtained.

6-Oxa-3-homo-5∝-cholestane-3,7-dione (CCLXIV)

The hydroxy lactone (CCLIX) on Jones' oxidation[71] provided a compound, m.p. 191° which analysed for $C_{27}H_{44}O_3$ and was identified as the oxolactone (CCLXIV). Its i.r. spectrum showed bands at 1732s (∈ -lactone), 1720s (C=O) and 1040 cm^{-1} (C-O).

3-Oxo-5,6-secocholest-4-en-6-oic acid (CCLXVI)

It was considered possible that base hydrolysis of the lactone (CCLXIV) might provide the α,β-unsaturated oxo-secoacid (CCLXVI) via the intermediate (CCLXV) which will be initially formed. As (CCLXV) will be a typical β-ketol, instant loss of water might prevail over relactonization to eventually give(CCLXVI). The reaction followed the expected course and gave (CCLXVI) as a noncrystallizable oil which analysed for $C_{27}H_{44}O_3$ and was characterized as the secoacid (CCLXVI) on the basis of its u.v. and i.r. spectrum. Its u.v. spectrum exhibited absorption maxima at 230 nm (ε 10000) which is a clear indication of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum gave bands at 3550-3200br (CO-OH), 1725s (CO-OH), 1675s (C=C-C=O) and 1610 cm^{-1}(C=C).

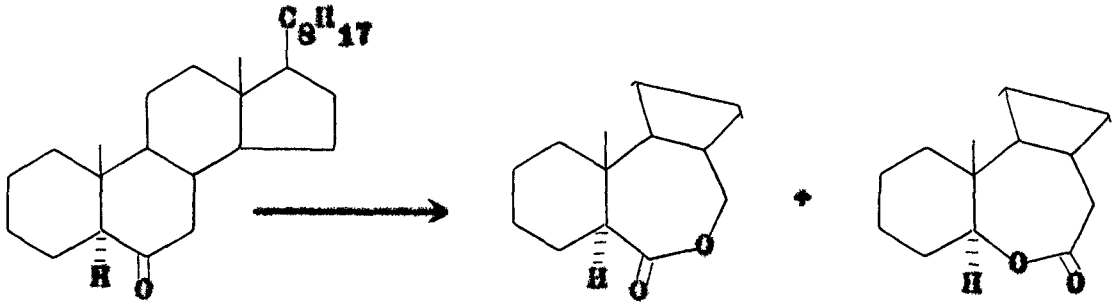
Methyl 3-oxo-5,6-secocholest-4-en-6-oate (CCLXVII)

To obtain further support in favour of the secoacid (CCLXVI) it was converted to its methyl ester (CCLXVII) by reaction with diazomethane. The methyl ester (CCLXVII) analysed for $C_{28}H_{46}O_3$ and showed λ max at 230 nm (ε 9860) for the α,β-unsaturated carbonyl chromophore. The i.r. spectrum showed bands at 1735s (CO-OCH_3), 1680s (C=C-C=O), 1615 (C=C)and 1190 cm^{-1} (methyl ester). Its n.m.r. spectrum gave a clear doublet for 1 proton at δ 6.75 which is ascribable to a vinylic proton (C5-H, J 10 Hz). This downfield shift is compatible with its being β- to a carbonyl group.

Another doublet integrating for 1 proton was observed at δ 5.68 (C4-$\underline{H}$, J 10 Hz). A sharp singlet for 3 protons appeared at δ 3.6 (COOC$\underline{H}_3$). A multiplet at δ 2.51 integrating for 4 protons has been assigned to C2-$\underline{H}_2$ and C7-$\underline{H}_2$. Other methyl signals appeared at δ1.2 (C10-C$\underline{H}_3$), 0.66 (C13-C$\underline{H}_3$), 0.9 and 0.8 (other methyl groups).

Baeyer-Villiger oxidation of 5α-cholestan-6-one (XXXIII)

The ketone (XXXIII) on perbenzoic acid oxidation in the usual manner also afforded two compounds, m.pts. 126° and 155°.



(XXXIII) (CCXLVIII) (XXXV)

Characterization of the compound, m.p. 126° as 7-oxa-B-homo-5α-cholestan-6-one (CCXLVIII)

The compound, m.p. 126° analysed for $C_{27}H_{46}O_2$ and this composition was supported by its mass spectrum which gave molecular ion peak at m/e 402 ($C_{27}H_{46}O_2$). The compound, m.p. 126° is characterised as 7-oxa lactone (CCXLVIII) on the basis of its

spectral analogy to the lactone (CCLV). Its i.r. spectrum showed bands at 1722s (ϵ -lactone), 1135 and 1080 cm^{-1}(C-O). The n.m.r. spectrum gave a broad signal for 1 proton at δ 4.26 (C7a-βH) and a doublet integrating for 1 proton at δ 4.16 (C7a-αH, J 3.5 Hz). A doublet of doublet for 1 proton appeared at δ 2.66 (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 5 Hz). Other signals were observed at δ 0.9, 0.8 and 0.7 (5 methyl groups).

Characterization of the compound, m.p. 155° as 6-oxa-8-homo-5α-cholestan-7-one (XXXV)

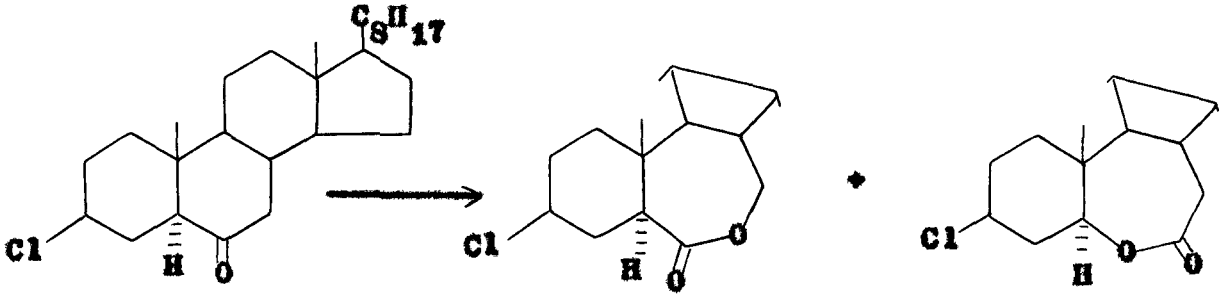
The compound, m.p. 155° analysed for $C_{27}H_{46}O_2$ and this composition was further supported by molecular ion peak at m/e 402 ($C_{27}H_{46}O_2$) in its mass spectrum. The compound, m.p. 155° showed spectral similarity to the lactone (CCLVI) and on this basis was easily characterised as the 6-oxa lactone (XXXV). Its i.r. spectrum gave bands at 1720s (ϵ -lactone), 1035 cm^{-1} (C-O). In n.m.r. spectrum a doublet of doublet for 1 proton was seen at δ 4.16 (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 5 Hz). A broad singlet for 1 proton was observed at δ 2.5 (C7a-βH) and a doublet was found at δ 2.41 (C7a-αH, J 3.5 Hz). Other signals were observed at δ 0.9, 0.8 and 0.7 (5 methyl groups).

It may be pointed that the lactones (XXXV) and (CCXLVIII) were obtained in the ratio of 75:25, whereas in the former cases described earlier, ratio between 6-oxa and 7-oxa isomers remained

about 1:1. Fonken and Miles[13] reported to have obtained only the 6-oxa isomer (XXXV) from this reaction and reported its m.p. to be 143-44°. An admixture of the isomeric lactones (CCXLVIII) and (XXXV) in equal amount, melted at 112°. But when mixed in the ratio of the yield (1:3) of respective lactones it melted at 142-44°, coinciding with the m.p. reported[13] for (XXXV). It therefore appears that the then product of Fonken and Miles was actually a mixture of the isomeric lactones (CCXLVIII) and (XXXV) in the ratio of 1:3.

Baeyer-Villiger oxidation of 3β-chloro-5∝-cholestan-6-one(CCXLIX)

The chloroketone (CCXLIX) on similar treatment with perbenzoic acid and subsequent column chromatography over silica gel gave two compounds, m.pts. 145° and 185° roughly in equal proportion.



(CCXLIX) (CCLXVIII) (CCXLIII)

Characterisation of compound, m.p. 145° as 3β-chloro-7-oxa-B-homo-5∝-cholestan-6-one (CCLXVIII)

The compound, m.p. 145° analysed for $C_{27}H_{45}O_2Cl$ and this composition was supported by molecular ion peaks at m/e 436/438(3:1)

($C_{27}H_{45}O_2Cl$) in its mass spectrum. The compound m.p. 145° was characterized as 7-oxa lactone (CCLXVIII) by its spectral properties and chemical transformation. The i.r. spectrum gave bands at 1715s (ϵ-lactone), 1195, 1085 (C-O) and 735 cm^{-1}(C-Cl). The n.m.r. spectrum gave a broad singlet for 1 proton at δ 4.09 (C7a-βH), and a doublet for 1 proton at δ 4.0 (C7a-αH, J 5 Hz). A broad signal integrating for 1 proton was observed at δ 3.7 ($W_{\frac{1}{2}}$ 14 Hz; C3-αH). A doublet of doublet for 1 proton was seen at δ 2.85 (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz). Other signals were observed at δ 0.9, 0.8 and 0.7 (3 methyl groups).

The compound m.p. 145° on sodium-pentyl alcohol reduction afforded the 7-oxa lactone (CCXLVIII), this conversion further supports the 7-oxa assignment to the compound, m.p. 145°.

Characterisation of the compound, m.p. 185° as 3β-chloro-6-oxa-B-homo-5α-cholestan-7-one (CCXLIII)

The compound, m.p. 185° analysed for $C_{27}H_{45}O_2Cl$ and this composition was supported by its mass spectrum which showed molecular ion peaks at m/e 436/438 (3:1)($C_{27}H_{45}O_2Cl$). The compound, m.p. 185° is characterised as the 6-oxa-chlorolactone (CCXLIII) on the basis of its spectral properties and chemical transformation. Its i.r. spectrum showed bands at 1718s (ϵ-lactone), 1045 (C-O) and 740 cm^{-1} (C-Cl). The n.m.r. spectrum gave a doublet of doublet for 1 proton at δ 4.21 (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz). A broad

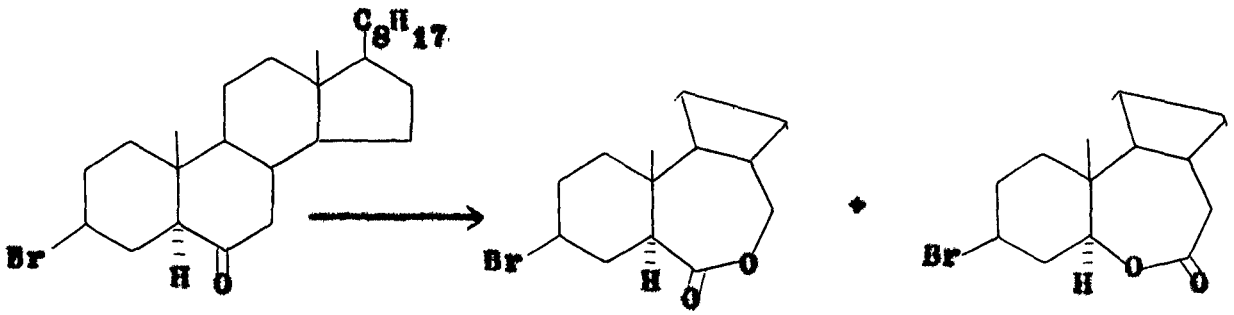
signal for 1 proton was observed at δ 3.66 ($W\frac{1}{2}$ 14 Hz; C3-αH). A broad singlet integrating for 1 proton was seen at δ 2.5 (C7a-βH) and a doublet for 1 proton at δ 2.41 (C7a-αH, J 5 Hz). Other signals were observed at δ 0.9, 0.8 and 0.7 (3 methyl groups).

The chlorolactone, m.p. 185° on sodium-pentyl alcohol reduction furnished the 6-oxalactone (XXXV), thus further supporting 6-oxa-structure for (CCXLIII).

Previously, the m.p. of (CCXLIII) was reported[69] to be 167-68° which differs from the one observed in the present study. From the m.p. range (158-64°) of an admixture of isomeric lactones (CCLXVIII) and (CCXLIII), which is very close to the m.p. of (CCXLIII) reported earlier, there appears to be no doubt that the reported lactone (CCXLIII) was, in fact, a mixture of the isomeric lactones (CCLXVIII) and (CCXLIII).

Baeyer-Villiger oxidation of 3β-bromo-5α-cholestan-6-one (CCL)

The bromoketone (CCL) on perbenzoic acid oxidation in the usual manner afforded two compounds, m.pts. 171° and 183°.



(CCL) (CCLXIX) (CCXLIV)

Characterization of compound, m.p. 171° as 3β-bromo-7-oxa-B-homo-5α-cholestan-6-one (CCLXIX)

The compound, m.p. 171° analysed for $C_{27}H_{45}O_2Br$ and was characterized as 7-oxa lactone (CCLXIX) by its spectral properties. The i.r. spectrum gave bands at 1712s (∈ -lactone), 1190, 1080(C-O) and 720 cm^{-1} (C-Br). Its n.m.r. spectrum gave a broad signal integrating for 1 proton at δ 4.1 (C7a-β-H), and a doublet for 1 proton at 4.01 (C7a-α-H, J 5 Hz). A broad signal for 1 proton appeared at δ 3.75 ($W_{\frac{1}{2}}$ 14 Hz; C3-α-H). A doublet of doublet integrating for 1 proton was seen at δ 2.64 (C5-α-H, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz). Other signals were observed at δ 0.9, 0.8 and 0.7 (5 methyl groups).

The compound,m.p. 171° on sodium-pentyl alcohol reduction gave the 7-oxa lactone (CCXLVIII). This conversion further supports the 7-oxa assignment to the compound, m.p. 171°.

Characterization of the compound, m.p. 183° as 3β-bromo-6-oxa-B-homo-5α-cholestan-7-one (CCXLIV)

The compound, m.p. 183° analysed for $C_{27}H_{45}O_2Br$ and is characterized as the 6-oxa-bromo lactone (CCXLIV) on the basis of its spectral analogy to the 6-oxa lactone (CCLVI). Its i.r. spectrum showed bands at 1715s (∈ -lactone), 1035 (C-O) and 720 cm^{-1} (C-Br). The n.m.r. spectrum gave a doublet of doublet for 1 proton at δ 4.2 (C5-α-H, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz). A broad signal integrating

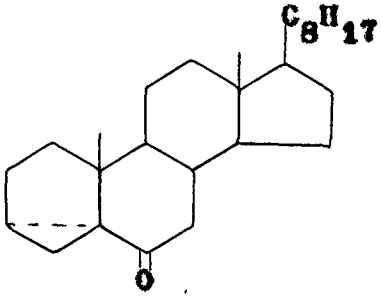
for 1 proton was observed at δ 3.70($W\frac{1}{2}$ 14 Hz; C3-αH). A broad singlet for 1 proton was seen at δ 2.51 (C7a-βH) and a doublet for 1 proton at 2.41 (C7a-αH, J 5 Hz). Other signals were observed at δ 0.9, 0.8, and 0.7 (5 methyl groups).

The compound, m.p. 183° on sodium-pentyl alcohol reduction afforded the 6-oxa lactone (XXXV) thus provided additional evidence in favour of its assignment as 6-oxabromo lactone (CCXLIV).

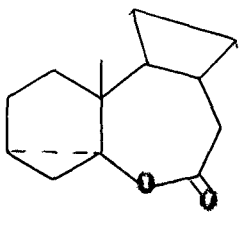
Previously, the m.p. of (CCXLIV) was reported[69] to be 179° which differs from the one reported here. A mixture of (CCLXIX) and (CCXLIV) in the ratio of their respective yield melted at 177-79° corresponding to the m.p. reported for (CCXLIV). It clearly indicates that the earlier reported lactone (CCXLIV) was a mixture of (CCLXIX) and (CCXLIV).

Baeyer-Villiger oxidation of 3α,5-cyclo-5α-cholestan-6-one (CCX)

The ketone (CCX) on perbenzoic acid oxidation provided only a single compound, m.p. 123-27° which was found identical with the previously reported lactone (CCXLV)[69]. Its t.l.c. and i.r. spectrum were also found identical with the previously obtained 6-oxa lactone (CCXLV).



(CCX)



(CCXLV)

From the present study it becomes obvious that the migratory aptitude of groups in Baeyer-Villiger oxidation is not as simple as was believed, in the sense that a more highly substituted carbon migrates preferentially than a less substituted carbon. From the Table-III which records the yield of various 6-oxa and 7-oxa compounds observed in the present study, it is clear that the C3 substituent could well have an influence on the course of the reaction. In general, though, the migratory power of alkyl groups in Baeyer-Villiger oxidation shows a descending trend in the order, tertiary, secondary, primary and methyl, this is not a rule. Often departures have been observed which are a consequence of steric and electronic factors. The tendency of various groups to migrate to an electron deficient oxygen in Baeyer-Villiger oxidation has been shown to bear a direct relationship with its intrinsic ability to support a positive charge in the transition state[72].

Table - III

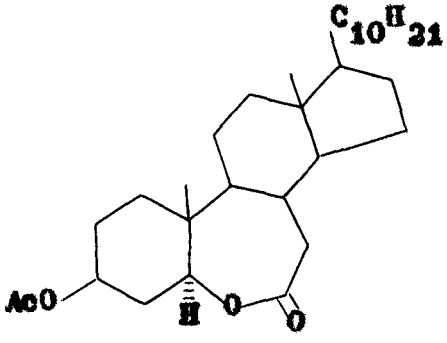
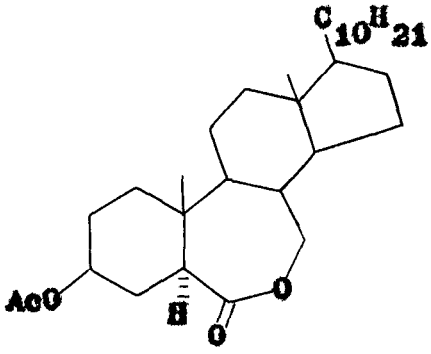
6-Oxaisomers	Yield(%)	7-Oxaisomers	Yield(%)
 (CCLVI)	49	 (CCLV)	51

Table-III(Contd.)

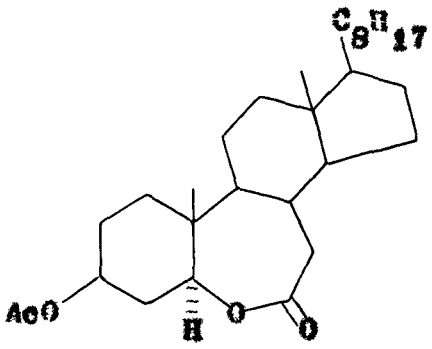
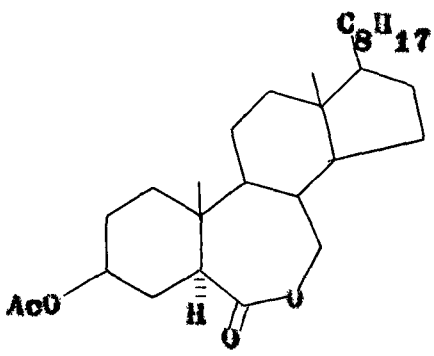
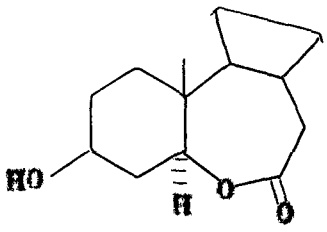
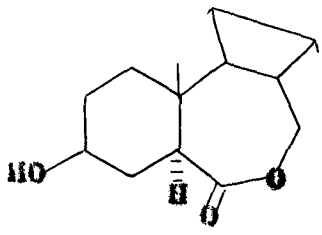
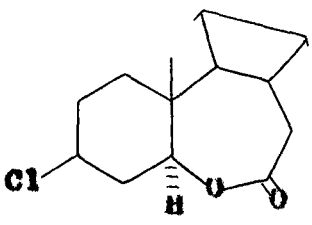
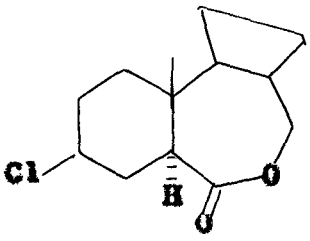
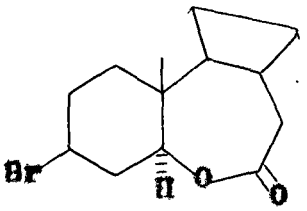
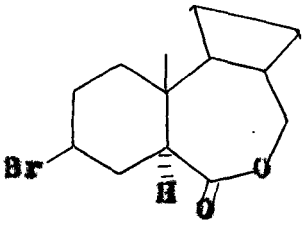
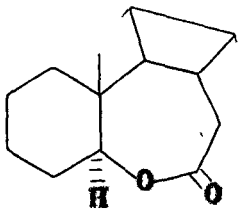
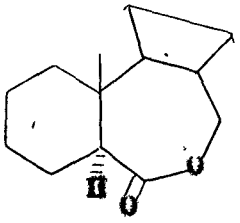
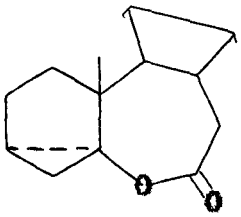
6-Oxaisomers	Yield(%)	7-Oxaisomers	Yield(%)
 (XXXVI)	49.7	 (CCLVII)	50.3
 (CCLIV)	49.3	 (CCLVIII)	50.7
 (CCXLIII)	49.6	 (CCLXVIII)	50.4

Table-III(Contd.)

6-Oxaisomers	Yield(%)	7-Oxaisomers	Yield(%)
 (CCXLIV)	44.9	 (CCLXIX)	55.1
 (XXXV)	75.2	 (CCXLVIII)	24.8
 (CCXLV)	100		

Taking conformational[72] factors into consideration, it may be argued that transition state for oxidation may get sterically crowded by substituents at C3 resulting into diminished migration of C5 which indeed is the case in going from unsubstituted to substituted C6-ketosteroids. The bulk of the C3 substituent seems to have a more pronounced effect on the preferred migratory aptitude of C7 in relation to C5 as is evidenced by the behaviour of the chloro (CCXLIX) and the bromo (CCL) ketones towards perbenzoic acid. To some extent the electronic effects of these groups might also be in play. The exception is provided by (CCX) where only C5 migrates presumably due to its quarternary nature. Also, there is a strong likelihood of a better support of the positive charge in the transition state by the partial double bond nature of the cyclopropane moiety. This suggestion finds support from the observation that the peracid oxidation of conjugated enones almost invariably leads to enol lactones[32] rather than α,β-unsaturated lactones by preferential migration of a vinylic carbon. In conclusion, the Baeyer-Villiger oxidation of 6-ketosteroids may not stereospecifically provide only the 6-oxasteroids as revealed by the present study which redefines past conclusion.

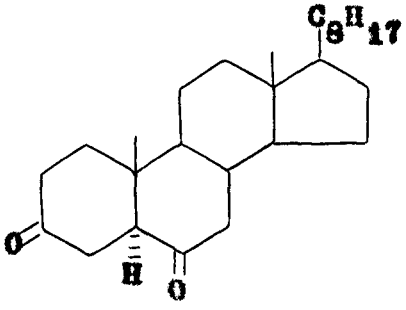
The Baeyer-Villiger oxidation of 5∝-cholestane-3,6-dione (CCXV)

Our study of the Baeyer-Villiger oxidation of 5∝-cholestane-3,6-dione (CCXV) was taken up for two reasons: (i) to obtain hitherto unreported steroidal bislactones and (ii) to clear a long standing ambiguity in chemical literature. This reaction had been studied by Windaus[73] in 1904 but the resultant product could not be characterized as even the work on structure determination of cholesterol had not been initiated at that time.

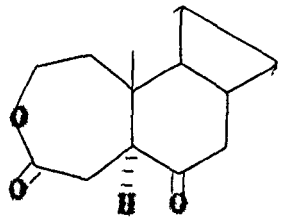
5∝-Cholestane-3,6-dione (CCXV) when treated with varying concentrations of perbenzoic acid invariably afforded a single product, m.p. 220°.

Characterization of the compound, m.p. 220° as 3-oxa-5∝-A-homocholestane-4,6-dione (CCLXX)

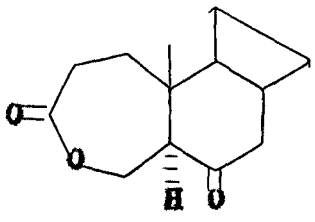
The compound, m.p. 220° analysed for $C_{27}H_{44}O_3$ which implied the insertion of only one oxygen atom during the course of the reaction. Introduction of a single oxygen leads to several possible isomeric monolactones such as (CCLXX), (CCLXXI), (CCLXII) and (CCLXIV).



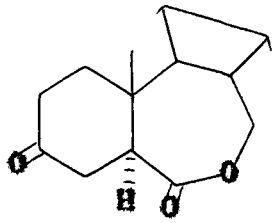
(CCXV)



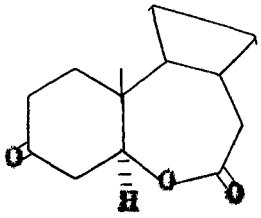
(CCLXX)



(CCLXXI)



(CCLXII)



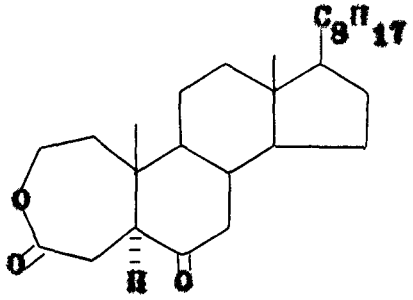
(CCLXIV)

During our reinvestigations of the peracid oxidation of 6-ketosteroids, we had prepared lactones (CCLXII) and (CCLXIV). The compound, m.p. 220° was different from these lactones and this helped us to eliminate structures (CCLXII) and (CCLXIV) for the compound m.p. 220°. The i.r. spectrum of compound m.p. 220° gave bands at 1735s (ϵ-lactone), 1700s (C=O) and 1270 cm^{-1} (C-O). These i.r. values are in agreement with both the structures (CCLXX) and (CCLXXI). The conclusive evidence in favour of (CCLXX) was obtained from n.m.r. spectrum. A broad multiplet for two protons at δ 4.27 is ascribable to a methylene attached to ester oxygen and its multiplicity could only result from its splitting by an adjacent methylene group. Thus the grouping ($O-CH_2-CH_2-$) present only in (CCLXX) excluded the alternate structure (CCLXXI) where the grouping ($-O-CH_2-CH-$) would exhibit lesser multiplicity. A multiplet spread between 3.15 to 2.3 integrating for 5 protons was assigned to methylene protons, C4a-$\underline{H}_2$, C7-$\underline{H}_2$ and methine proton C5-$\underline{H}$, which further supported the structure (CCLXX). Other signals were seen at δ 1.21(C10-CH_3), 0.68 (C13-CH_3), 0.92 and 0.86 (other methyl groups).

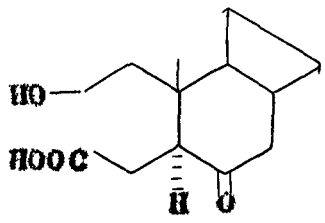
The noteworthy feature of this reaction is the formation of only the monolactone (CCLXX) and none of either the alternative monolactones or the dilactones envisagable. It appears that steric features determine the course of reaction. The C3-keto group being more exposed reacts dominantly over relatively hindered C6-keto function.

Hydrolysis of the lactone (CCLXX): 6-oxo-2,3-seco-2-hydroxy-5α-cholestan-3-oic acid (CCLXXII)

In order to support the structure (CCLXX) chemically the compound, m.p. 220° was subjected to alkaline hydrolysis which gave a compound, m.p. 188-90° which analysed for $C_{27}H_{46}O_4$. Its i.r. spectrum gave bands at 3400-3200br (COOH and OH), 1710s, and 1700sh (COOH and C=O). These values are compatible with the seco acid (CCLXXII). Windaus[73] in a similar study had isolated a compound with m.p. 185-217°.



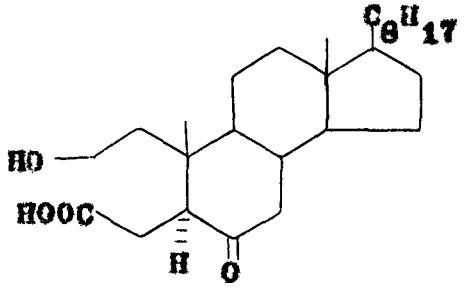
(CCLXX)



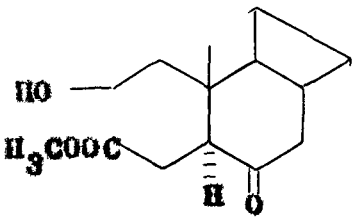
(CCLXXII)

Methyl 6-oxo-2,3-seco-2-hydroxy-5α-cholestan-3-oate (CCLXXIII)

To substantiate the presence of a carboxylic function in the compound (CCLXXII), it was treated with diazomethane which afforded the methyl ester (CCLXXIII), m.p. 90°. The compound m.p. 90° analysed for $C_{29}H_{48}O_4$ and was characterized as the methyl ester (CCLXXIII). The i.r. spectrum gave bands at 3500br (OH), 1730s, 1710s ($\underline{CO}$-OCH_3 and C=O) and 1190 cm^{-1} (methyl ester). The n.m.r. spectrum of (CCLXXIII) gave a singlet for 3 protons at δ 3.63 ascribable to $COOC\underline{H}_3$. Another multiplet centred at this point integrating for 2 protons has been assigned to hydroxyl bearing methylene group. α-Methylene protons and methine proton were spread between δ 3.2 to 2.15. Other signals were observed at δ 1.23 (C10-CH_3), 0.65 (C13-CH_3), 0.88 and 0.8 (other methyl groups).



(CCLXXII)



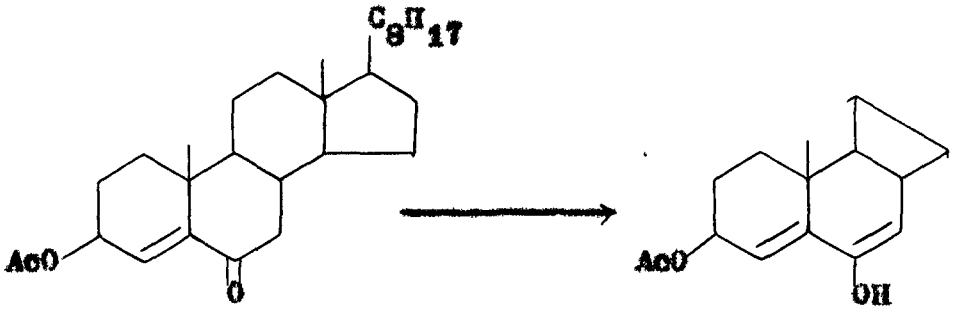
(CCLXXIII)

It is pertinent to mention here that at the beginning of the present century, Windaus had attempted to oxidize "cholestandione" (CCXV)[73] by heating at 70-75° for 3 hours, an acetic acid solution

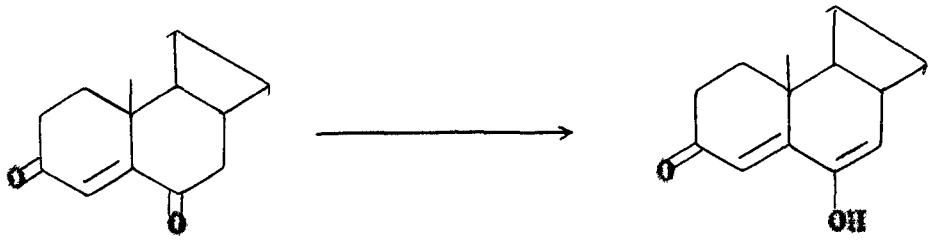
of (CCXV) and an aqueous solution of ammonium persulphate. This was done much before the correct structure of cholesterol was known. With the nuclear location of the two oxo groups unknown, Windaus suggested the reaction product to be an "oxyketo carbonic acid" (m.p. 185-217°) which could be converted to its methyl ester (m.p. 105°). He expressed surprise at the response of only one ketone towards oxidizing agent with the other ketone intact. From our observations it seems likely that in the hands of Windaus, reaction followed a course analogous to ours with the difference that it went beyond the primary oxidation product (CCLXX) and yielded the hydroxy acid (CCLXXII) under their reaction conditions.

B. <u>α,β-Unsaturated Ketones</u>

In continuation of our work in the area of Baeyer-Villiger oxidation of steroidal α,β-unsaturated ketones, it was considered of interest to study the perbenzoic acid oxidation of 3β-acetoxy-cholest-4-en-6-one (CCLIV) and cholest-4-ene-3,6-dione (CCXXI). The selection of these substrates was largely governed by two considerations: (a) their accessibility and (b) anticipation of some unusual results. In view of the functionalities present in (CCLIV) and (CCXXI) these have strong tendency for enolization such as (CCLIV $\longrightarrow$ CCLIV') and (CCXXI $\longrightarrow$ CCXXI') and this fact was considered worth exploitation. From the subsequent discussion it will become obvious that this study was amply awarded through formation of several interesting compounds.



(CCLIV) (CCLIV')



(CCXXI) (CCXXI')

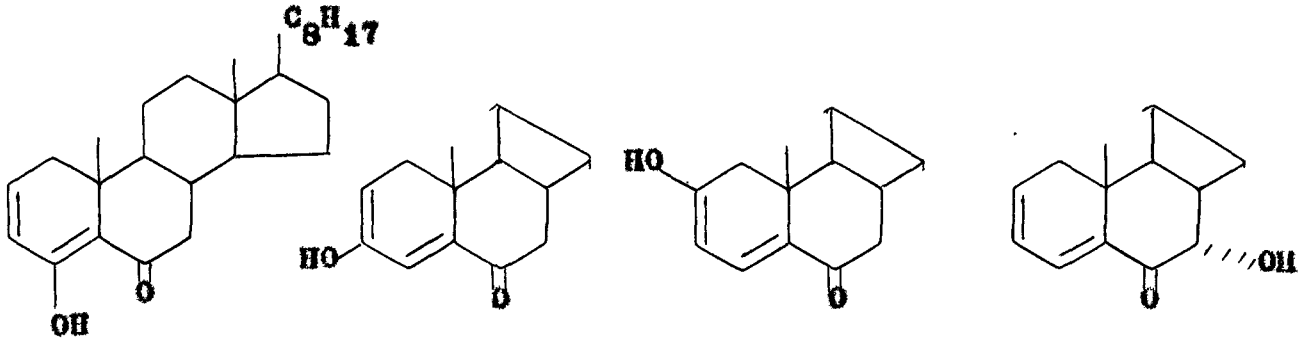
Reaction of 3β-acetoxycholest-4-en-6-one (CCLIV) with perbenzoic acid

Oxidation of 3β-acetoxycholest-4-en-6-one (CCLIV) with 1 mole equivalent of perbenzoic acid (p-toluenesulphonic acid monohydrate as catalyst) gave four compounds, m.pts. 95°, 165°, 190° and a noncrystallizable oil. When treated with an excess of perbenzoic acid (2.5 mole equivalent), (CCLIV) provided in addition to the compound, 190°, and noncrystallizable oil, another product, m.p. 245°.

Characterization of the compound, m.p. 95°, as 4-hydroxycholesta-2, 4-dien-6-one (CCLXXIV)

The compound, m.p. 95° analysed for $C_{27}H_{42}O_2$. Its u.v. spectrum[74] showed absorption maxima at 320 nm (ε 7700) which suggested a dienone like chromophore. This was further supported by i.r. spectrum[75], which showed prominent bands at 1660s and 1620 cm^{-1} compatible with C=C-C=C-C=O and C=C-C=C- chromophores,

respectively; other significant bands were observed at 3380br (OH) and 1100 cm^{-1}(C-O). On the basis of the composition and spectral values of the compound, m.p. 95°, several possible structures (CCLXXIV - CCLXXIV"') may be suggested for it. The n.m.r. spectrum clearly supported the structure (CCLXXIV). A multiplet at δ 6.66 for 1 proton is ascribable to C2-H. Another multiplet at 6.0 (2H) can be assigned to C3-H and hydroxyl proton (OH) since on deuterium exchange this signal was reduced and modified into a distorted doublet with major J 10 Hz, a clear evidence of the vicinal coupling of vinylic C3-H with vinylic C2-H. The distortion of doublet might be a result of long range allylic coupling of C3-H with C1-H_2. In the alternate structure (CCLXXIV'), a singlet

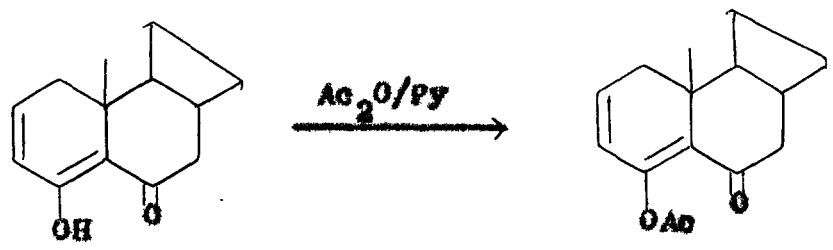


(CCLXXIV) (CCLXXIV') (CCLXXIV") (CCLXXIV"')

is likely to be observed for C4-H. In (CCLXXIV"), a doublet each for C3-H and C4-H and in (CCLXXIV"'), three vinylic protons are expected to be seen. A multiplet integrating for 2 protons was observed at δ 2.7 and was assigned to C7-H_2. Other signals were seen at δ 1.08 (C10-CH_3), 0.61 (C13-CH_3), 0.81 and 0.71 (other methyl groups).

4-Acetoxycholesta-2,4-dien-6-one (CCLXXV)

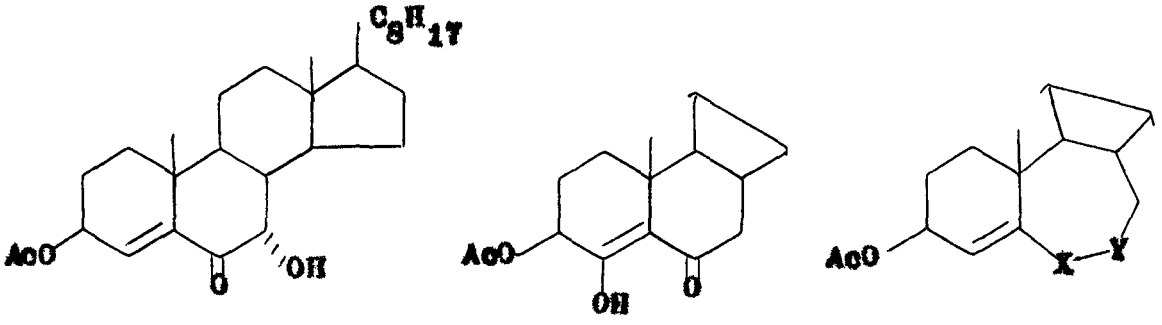
Acetylation of the hydroxy compound (CCLXXIV) afforded a noncrystallizable oil. The oil analysed for $C_{29}H_{44}O_3$ and was characterized as the enol acetate (CCLXXV). Its u.v. spectrum gave absorption maxima at 315 nm (ϵ 7209) indicating the presence of a dienone like chromophore. The i.r. spectrum[75] gave bands at 1770s (enol acetate), 1210s (acetate), 1100 and 1050 cm^{-1}(C-O).



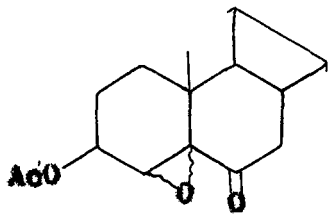
(CCLXXIV) (CCLXXV)

Characterization of the compound, m.p. 165° as 6-oxo-7α-hydroxy cholest-4-en-3β-yl acetate (CCLXXVI)

The compound, m.p. 165° analysed for $C_{29}H_{46}O_4$. This composition suggested addition of one oxygen atom to the substrate, which leads to (CCLXXVI) as well as its isomeric structures (CCXLVII) and (CCLXXVIa-CCLXXVIc).



(CCLXXVI) (CCLXXVIa) (CCXLVII) X,O; Y,CO
CCLXXVIb) X,CO; Y,O



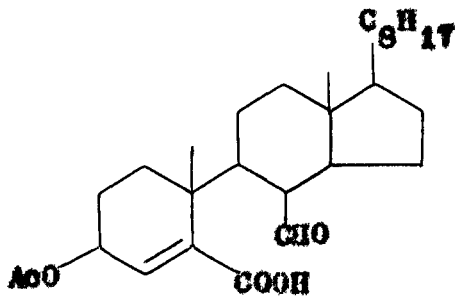
(CCLXXVIc)

The u.v. spectrum gave absorption maxima at 238 nm(ϵ 6200) which indicated the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum gave bands at 3380br (OH), 1740s (CH_3-CO-O), 1690s (C=C-C=O), 1645 (C=C), 1235 (acetate) and 1050 cm^{-1} (C-O). On the basis of i.r. spectrum, structures (CCXLVII), (CCLXXVIb) and (CCLXXVIc) can be discarded since none of these possess a hydroxy function. Moreover, (CCLXXVI) was different from an authentic sample[79] of (CCXLVII). Thus a choice between the structures (CCLXXVI) and (CCLXXVIa) had to be made. A distinction between (CCLXXVI) and (CCLXXVIa) became possible from n.m.r. spectrum wherein a distorted doublet like signal at δ 6.08 (1H, major J 2 Hz) could be ascribed to C4-H (long range coupling with C2-H, M pattern). In (CCLXXVIa), no signal for vinylic proton will be observed. A broad signal integrating for 1 proton appeared at δ 5.37 ($W_{\frac{1}{2}}$ 12 Hz) which has been ascribed to C3-αH. The signal for C3-αH (axial) appeared at a relatively lower field and this could be attributed to its being allylic to the C4, C5 double bond. A broadened singlet like signal at δ 3.27 for 1 proton is ascribable to C7-βH. Theoretically this signal would be expected to be a doublet by splitting with C8-βH. An

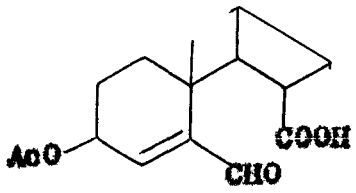
examination of the Dreiding model of (CCLXXVI) showed the dihedral angle between C7-βH (equatorial) and C8-βH (axial) to be almost 90° which may account for the absence of sharp splitting of this signal. A broad signal for 1 proton was observed at δ 2.41 which disappeared on D_2O addition and was assigned to C7-OH. A sharp singlet for 3 protons appeared at δ 2.05 (CH_3COO-). Other signals were seen at δ 1.01 (C10-CH_3), 0.61 (C13-CH_3), 0.81 and 0.71 (other methyl groups).

Characterization of the compound, m.p. 190° as 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII)

The compound, m.p. 190° analysed for $C_{29}H_{46}O_5$. From the molecular composition it is evident that two oxygen atoms have been added to the parent ketone during the reaction. The u.v. spectrum gave absorption maxima at 209 nm (ϵ 7800) indicating the presence of an α,β-unsaturated carbonyl chromophore, preferably a carboxylic keto conjugated with a carbon-carbon double bond. Its i.r. spectrum showed the presence of a conjugated carboxylic acid, aldehyde and acetate functions and an olefinic linkage. The i.r. bands were obtained at 3400-3200br (CO-OH), 2725w (H-C=O), 1740s (CH_3-COO), 1720s (HC=O), 1690s (C=C-COOH), 1645 (C=C) and 1240 cm^{-1}(acetate). Both (CCLXXVII) and its positional isomer (CCLXXVIII) contain these groups.



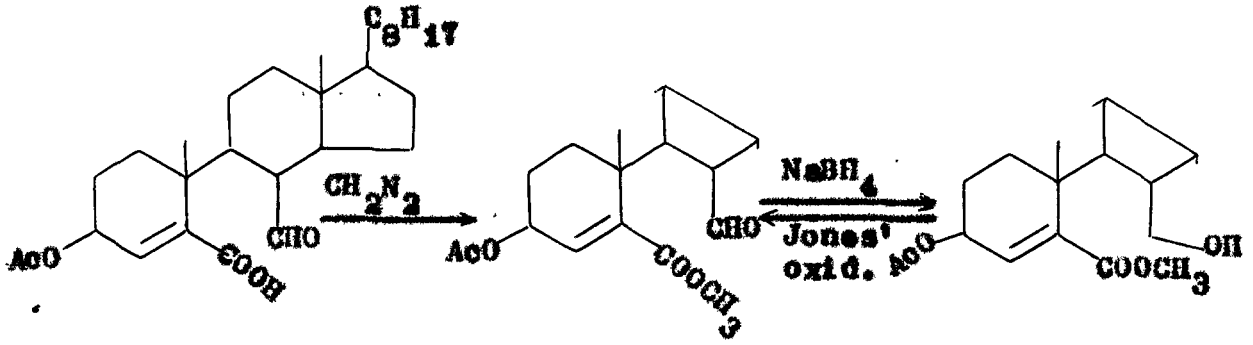
(CCLXXVII)



(CCLXXVIII)

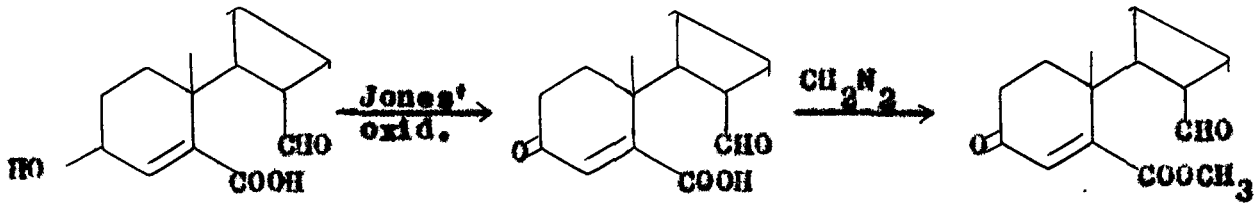
The n.m.r. spectrum was helpful in arriving at a definite conclusion where a distorted doublet at δ 9.55 integrating for 2 protons is ascribable to acid and aldehyde protons (COO<u>H</u> and <u>H</u>CO). On deuterium exchange of the acid proton, this signal was simplified to a neat doublet (J 5.5 Hz) of the aldehyde proton. This suggests that there is a methine proton in the vicinity of the aldehyde proton. Thus the grouping C<u>H</u>-C<u>H</u>O supports the structure (CCLXXVII) where C8-βH splits aldehyde proton, and excludes (CCLXXVIII) where such a possibility does not exist. An unresolved distorted doublet like signal at δ 6.9 (1H, major J 1.5 Hz) is ascribable to C4-vinylic proton. This downfield shift is compatible with its being β- to the acid carbonyl. A broad signal integrating for 1 proton appeared at 5.35 ($W\frac{1}{2}$ 12 Hz) which has been ascribed to C3-α<u>H</u>. The signal for C3-α<u>H</u> (axial) appeared at a relatively lowerfield and this could be attributed to its being allylic to the C4, C5 double bond. A multiplet for 1 proton appeared at δ 2.6 (C8-<u>H</u>). A sharp singlet integrating for 3 proton appeared at δ 2.07 and it is easily ascribable to acetate protons ($C\underline{H}_3$-COO-). Other signals were observed at δ 1.27 (C10-CH_3), 0.67 (C13-CH_3), 0.9 and 0.8 (other methyl groups).

In order to get additional information regarding the compound (CCLXXVII), the following reactions were carried out, which in a way also support the assigned structure for the compound, m.p. 190°.



(CCLXXVII) (CCLXXIX) (CCLXXX)

(CCLXXXI)



(CCLXXXII) (CCLXXXIII) (CCLXXXIV)

Interestingly, the aldehydic groups in (CCLXXXII) and (CCLXXIX) resisted oxidation by Jones' reagent.

Methyl 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oate (CCLXXIX)

The presence of a carboxylic function in the compound (CCLXXVII) was further substantiated by its conversion to the methyl ester (CCLXXIX), m.p. 150°. The methyl ester (CCLXXIX) analysed for $C_{30}H_{48}O_5$ and its u.v. spectrum gave absorption maxima at 210 nm (ϵ 7750). The i.r. spectrum showed bands at 2740m(H-C=O), 1745s (CH_3-COO), 1720s (HC=O and C=C-CO-OCH_3), 1645 (C=C), 1240 (acetate), and 1183 cm^{-1} (methyl ester). The n.m.r. spectrum showed a clear doublet at δ 9.43 (H-CO, J 6 Hz), an unresolved distorted doublet like signal at δ 6.58 (H-C4=C, major J 1 Hz) and a broad peak centred at δ 5.2 ($W_{\frac{1}{2}}$ 11 Hz) integrating for 1 proton was assigned to C3-<u>H</u>. A sharp singlet for 3 protons was observed at δ 3.7, which can be easily ascribed to -COO<u>CH</u>$_3$. A singlet at δ 2.0 integrating for 3 protons can be assigned to methyl group of an acetate function. Other signals were seen at δ 1.23 (C10-CH_3), 0.65 (C13-CH_3), 0.89 and 0.80 (other methyl groups).

Methyl 7-hydroxy-3β-acetoxy-6,7-secocholest-4-en-6-oate (CCLXXX)

The 7-oxo function of (CCLXXIX) was carefully reduced with sodium borohydride to furnish (CCLXXX) as a noncrystallisable oil which analysed for $C_{30}H_{50}O_5$. The u.v. spectrum gave absorption maxima at 210 nm (ϵ 7200) and its i.r. spectrum showed bands at

3500br (OH), 1740s ($CH_3\underline{CO}O$), 1720s (C=C-$\underline{CO}OCH_3$), 1635 (C=C), 1230 (acetate), 1190, 1065 and 1025 cm^{-1} (C-O). The weak peak at 2740 cm^{-1} characteristic of aldehyde function in (CCLXXIX) was not present in this spectrum which supported the absence of such a grouping in (CCLXXX). The n.m.r. spectrum of compound (CCLXXX) gave an unresolved distorted doublet like signal at δ 6.55 ($\underline{H}$-C4=C, major J 1 Hz), and a broad signal integrating for 1 proton was observed at δ 5.33 ($W\frac{1}{2}$ 12 Hz; C3-$\underline{H}$). A doublet for 2 protons appeared at δ 3.83 (J 5.5 Hz) which is ascribable to C7-$\underline{H}_2$. A sharp singlet integrating for 3 protons was observed at δ 3.7 and this peak was easily assigned to methyl ester protons ($COOC\underline{H}_3$). Acetate methyl protons appeared as a sharp singlet at δ 2.08 ($C\underline{H}_3COO$). The hydroxyproton was seen at δ 1.7 which disappeared on D_2O addition. Other signals were observed at δ 1.21 (C10-CH_3), 0.65 (C13-CH_3), 0.9 and 0.8 (other methyl groups).

Methyl 3β,7-diacetoxy-6,7-secocholest-4-en-6-oate (CCLXXXI)

The hydroxy compound (CCLXXX) on treatment with acetic anhydride-pyridine was converted to its acetate (CCLXXXI) obtained as an oil. The oil analysed for $C_{32}H_{52}O_6$ and its u.v. spectrum gave absorption maxima at 212 nm ($\leq$ 8000). The i.r. spectrum of compound (CCLXXXI) gave bands at 1740s (2 x $CH_3\underline{CO}$-O), 1720s (C=C-$\underline{CO}OCH_3$), 1635 (C=C), 1235 (acetate), 1190, 1060 and

1020 cm^{-1} (C-O). The n.m.r. spectrum of the compound (CCLXXXI) exhibited an unresolved distorted doublet like signal for 1 proton at δ 6.58 (HC4=C, major J 1 Hz) and a broad signal integrating for 1 proton appeared at δ 5.33 ($w_{\frac{1}{2}}$ 12 Hz; C3-H). A doublet for 2 protons appeared at δ 4.15 (C7-H_2, J 5 Hz) and the methoxy group of ester was seen as singlet at δ 3.76 ($COOCH_3$). A singlet integrating for 6 protons was observed at δ 2.05 which is easily ascribable to two acetate methyl groups (C3-$OOCCH_3$ and C7-$OOCCH_3$). Other signals were seen at δ 1.23 (C10-CH_3), 0.65 (C13-CH_3), 0.9 and 0.8 (other methyl groups).

3β-Hydroxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXXII)

The acetate group of (CCLXXVII) was hydrolysed to give the hydroxy compound (CCLXXXII), m.p. 195° which analysed for $C_{27}H_{44}O_4$ and its u.v. spectrum gave absorption maxima at 215 nm (7600). The i.r. spectrum of the compound (CCLXXXII) showed bands at 3500-3200br (COOH and OH), 2730w (H-C=O), 1720s (H-C=O), 1695s (C=C-COOH), 1640 (C=C) and 1065 cm^{-1} (C-O). The n.m.r. spectrum of the compound (CCLXXXII) gave a distorted doublet like signal at δ 9.46 integrating for 2 protons (COOH and H-CO). On D_2O shake, this signal was simplified to a clear doublet (J 5 Hz) integrating for 1 proton (CH - $\overset{O}{\overset{\|}{C}}$ - H). The C4-vinylic proton was observed in the form of an unresolved distorted doublet like signal at δ 6.96 (major J 1 Hz). A broad peak for 1 proton was observed at δ 5.83

which disappeared on addition of D_2O and it is easily ascribable to hydroxy proton (-O<u>H</u>). A broad peak for 1 proton centred at δ 4.35 ($W\frac{1}{2}$ 12 Hz) is ascribable to C3-α<u>H</u>. A multiplet for 1 proton was seen at δ 2.5 (C8-β<u>H</u>). Other signals were observed at δ 1.27 (C10-CH_3), 0.67 (C13-CH_3), 0.9, and 0.8 (other methyl groups).

<u>3,7-Dioxo-6,7-secocholest-4-en-6-oic acid (CCLXXXIII)</u>

The hydroxy compound (CCLXXXII) on Jones' oxidation[71] gave the compound (CCLXXXIII), m.p. 160° which analysed for $C_{27}H_{42}O_4$. Surprisingly it was noted that C7-oxo function remained unaffected during oxidation. The u.v. spectrum of compound (CCLXXXIII) showed absorption maxima at 238 nm (ε 11870) which clearly indicated the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum of compound (CCLXXXIII) gave bands at 3400-3200br (COO<u>H</u>), 2735w (<u>H</u>-C=O), 1720s (H-C=O), 1700s (C=C-C<u>O</u>OH), 1685s (C=C-C=O) and 1615 cm^{-1} (C=C). The n.m.r. spectrum of compound (CCLXXXIII) gave a clear doublet (J 5 Hz) for 1 proton at δ 9.3 which is easily ascribable to aldehydic proton (<u>H</u>-CO). Acid proton appeared as a broad signal at δ 7.89 which disappeared on D_2O addition. A singlet at δ 6.66 integrating for 1 proton is assignable to vinylic proton (C4-<u>H</u>). A multiplet for 3 protons was observed at δ 2.6 (C8-β<u>H</u> and C2-$\underline{H}_2$). Other signals were seen at δ 1.4 (C10-CH_3), 0.65 (C13-CH_3), 0.9 and 0.81 (other methyl groups).

Methyl 3,7-Dioxo-6,7-secocholest-4-en-6-oate (CCLXXXIV)

The compound (CCLXXXIII) on treatment with diazomethane afforded the methyl ester (CCLXXXIV), m.p. 125°, which analysed for $C_{28}H_{44}O_4$ and its u.v. spectrum showed absorption maxima at 239 nm (ϵ 11200). The i.r. spectrum of the compound (CCLXXXIV) gave bands at 2740w (H-C=O), 1725s (H-C=O), 1710s (C=C-COOCH$_3$), 1690s (C=C-C=O), 1620 (C=C) and 1180 cm^{-1} (methyl ester). Its n.m.r. spectrum gave a clear doublet (J 5 Hz) for 1 proton at δ 9.36 (H-CO). A singlet for vinylic proton appeared at δ 6.48 (C4-H) and a sharp singlet for methyl group of ester was observed at δ 3.81 (COOCH$_3$). A multiplet integrating for 3 protons appeared at δ 2.6 which is ascribable to methine and methylene protons (C8-βH and C2-H$_2$). Other signals were seen at δ 1.08 (C10-CH$_3$), 0.66 (C13-CH$_3$), 0.88 and 0.7 (other methyl groups).

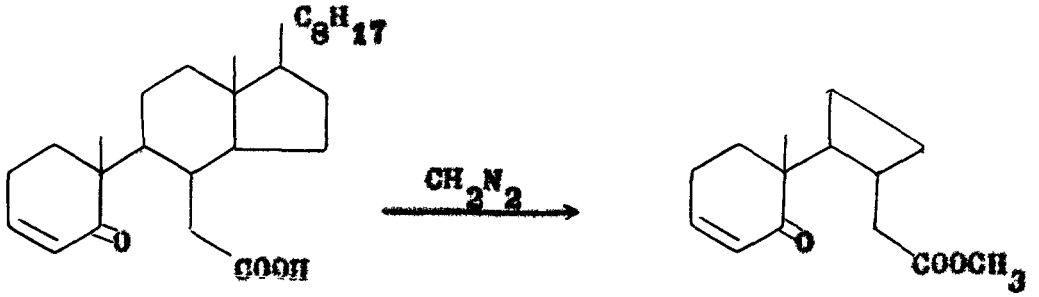
Characterization of the oil, as 5-oxo-5,6-secocholest-3-en-6-oic acid (CCLXXXV)

The oil analysed for $C_{27}H_{44}O_3$ and its u.v. spectrum gave absorption maxima at 230 nm (ϵ 10000), which clearly indicated the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum of the oil showed the presence of an acid function and was therefore easily characterized as the seco acid (CCLXXXV). In the infrared spectrum bands were obtained at 3440-3200br (COOH), 1710s (COOH), 1680s (C=C-C=O), and 1620 cm^{-1} (C=C); no bands for

an acetate group were observed, thus indicating that the acetate group got hydrolysed/eliminated during the course of the reaction. The n.m.r. spectrum of compound (CCLXXXV) gave signals at δ 8.77s (1H; exchangeable with deuterium, COO<u>H</u>), δ 6.75m (1H; C3-<u>H</u>; β - to a carbonyl group) and δ 5.87d (1H; J 10 Hz, C4-<u>H</u>). A multiplet at δ 2.7 is assignable to C7-<u>H</u>$_2$. No signal for the methyl group of an acetate function was observed. The other signals were observed at δ 1.08 (C10-CH$_3$), 0.66 (C13-CH$_3$), 0.88 and 0.7 (other methyl groups). It was further identified by direct comparison with an authentic sample[76].

<u>Methyl 5-oxo-5,6-secocholest-3-en-6-oate (CX)</u>

The secoacid (CCLXXXV) was converted to its methyl ester (CX) by treating the acid with diazomethane. The ester (CX) analysed for $C_{29}H_{46}O_3$ and its u.v. spectrum gave absorption maxima at 230 nm (ϵ 9977). The i.r. spectrum of the ester (CX) gave bands at 1735s (<u>COO</u>CH$_3$), 1675s (C=C-<u>C=O</u>), 1615 (C=C) and 1165 cm^{-1} (methyl ester). The n.m.r. spectrum of the ester (CX) gave signals at δ 6.75 integrating for 1 proton ascribable to C3-<u>H</u> (β- to a carbonyl group) and δ 5.8d (J 10 Hz) for 1 proton is easily ascribable to C4-<u>H</u>. A sharp singlet for 3 protons was observed at δ 3.57, which is clearly ascribable to methyl ester protons (COOC<u>H</u>$_3$). A multiplet at δ 2.8 was assigned to C7-<u>H</u>$_2$. Other signals were seen at δ 1.07 (C10-CH$_3$), 0.66 (C13-CH$_3$), 0.88 and 0.8 (other methyl groups). The ester (CX) was also found to be identical with its authentic sample[77].



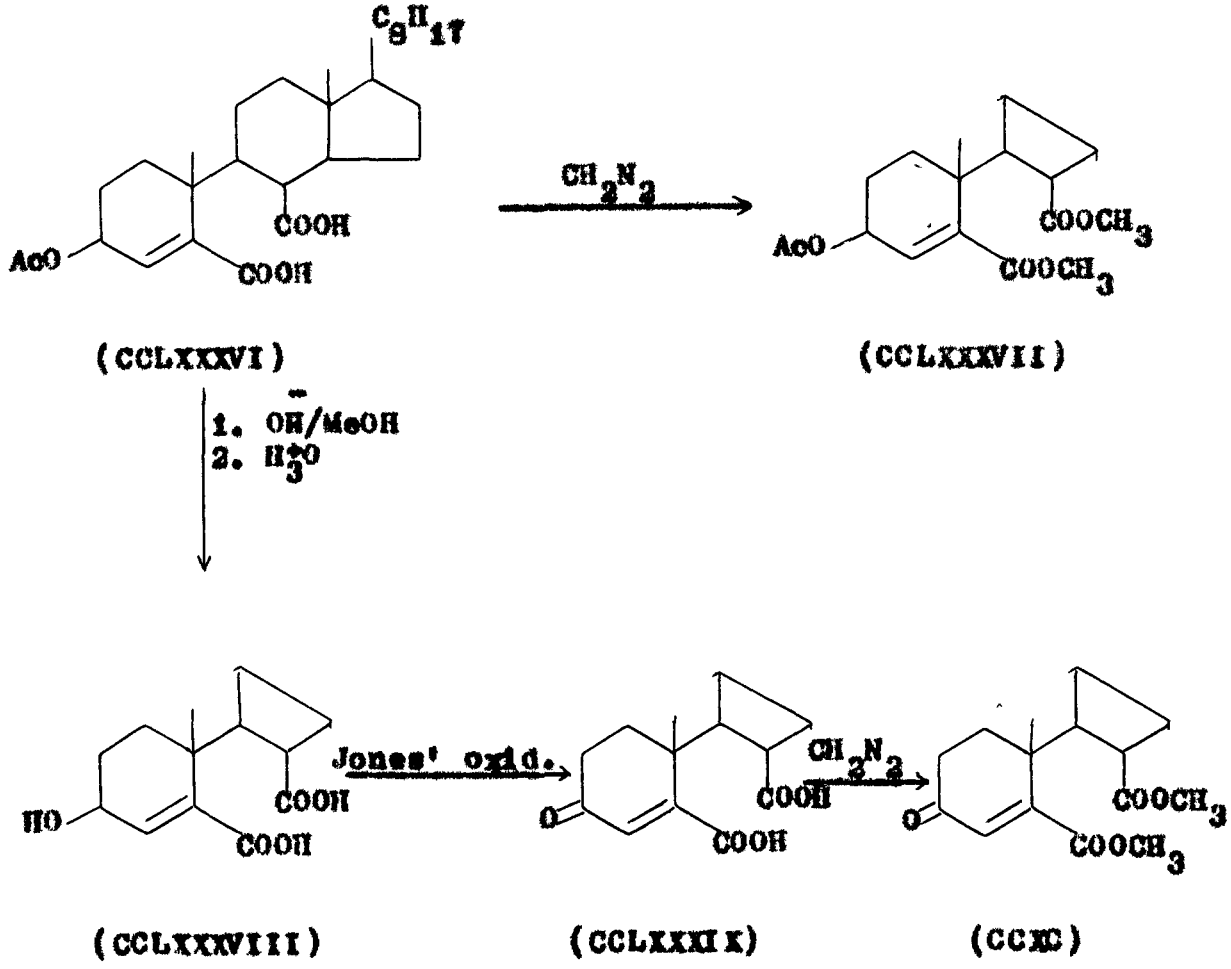
(CCLXXXV) (CX)

Characterization of the compound, m.p. 245°, as 3β-acetoxy-6,7-seecocholest-4-ene-6,8-dicarboxylic acid (CCLXXXVI)

The compound, m.p. 245° analysed for $C_{29}H_{46}O_6$ and thus showed an addition of three oxygen atoms to the starting ketone (CCLIV) during the course of reaction. Its u.v. spectrum gave absorption maxima at 212 nm (ϵ 7550) thus showing the presence of a conjugated carboxylic function. The i.r. spectrum of this compound showed bands at 3400-3200br (COOH), 1745s (CH_3-COO-), 1710s (COOH), 1690s (C=C-COOH), 1625 (C=C) and 1240 cm^{-1}(acetate). The weak band at 2740 cm^{-1} characteristic of aldehyde in (CCLXXVII) was not present in this spectrum. This observation suggested that the compound may be a dicarboxylic acid (CCLXXXVI). Evidence in favour of dicarboxylic acid structure was obtained from n.m.r. spectrum where two acid protons were seen at δ 7.4 and 7.35 and were found exchangeable with deuterium. It is to be noted that the peak due to chloroform impurity was observed at δ 7.3. An unresolved doublet like signal which was observed at δ 6.85 (1H, major J 1.5 Hz) has been assigned to C4-vinylic proton which is β - to carboxyl carbonyl. A broad signal at δ 5.41 ($W_{\frac{1}{2}}$ 12 Hz)

integrating for 1 proton has been ascribed to C3-αH (axial). A multiplet for 1 proton at δ 2.6 can be assigned to a methine proton (C8-βH). The methyl group of the acetate function appeared as a singlet at δ 2.1 (CH_3-COO-). Other signals were seen at δ1.45 (C10-CH_3), 0.68 (C13-CH_3), 0.91 and 0.83 (other methyl groups).

To further substantiate the structure (CCLXXXVI), the following transformations were made in order to obtain the known compounds (CCLXXXIX) and (CCXC). These transformations obviously support the structure (CCLXXXVI) for the compound, m.p. 245°.



(CCLXXXVI) (CCLXXXVII)

(CCLXXXVIII) (CCLXXXIX) (CCXC)

Dimethyl 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylate (CCLXXXVII)

The dicarboxylic acid (CCLXXXVI) was converted into the corresponding dimethyl ester (CCLXXXVII) with diazomethane. The dimethyl ester in its u.v. spectrum gave absorption maxima at 213 nm (ε 7500). Its i.r. spectrum gave bands at 1740s (CH_3-COO and $COOCH_3$), 1720s (C=C-$COOCH_3$), 1640 (C=C), 1240 (acetate) and 1185 cm^{-1} (C-O). In n.m.r. spectrum, an unresolved distorted doublet like signal at δ 6.63 (1H, major J 1 Hz) has been assigned to C4-vinylic proton. A broad signal at δ 5.38 ($W_{\frac{1}{2}}$ 12 Hz) integrating for 1 proton is ascribable to C3-αH (axial). The methyl groups of the methyl ester moieties appeared at δ 3.75s (C5-$COOCH_3$), 3.65s (C8-$COOCH_3$). A multiplet for 1 proton at δ 2.55 is ascribable to a methine proton (C8-βH). Methyl group of the acetate function was observed as a sharp singlet at δ 2.07 (CH_3-COO). Other signals were seen at δ 1.25 (C10-CH_3), 0.65 (C13-CH_3), 0.9 and 0.81 (other methyl groups).

3β-Hydroxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVIII)

The compound (CCLXXXVI) was hydrolysed to give the hydroxy compound (CCLXXXVIII), m.p. 225° which analysed for $C_{27}H_{44}O_5$ and its u.v. spectrum gave absorption maxima at 212 nm (ε 7450) indicating the presence of a conjugated carboxylic function. The i.r. spectrum of (CCLXXXVIII) showed bands at 3400-3100br (COOH

and OH), 1700s (COOH), 1695s (C=C-COOH), 1620 (C=C) and 1090 cm^{-1} (C-O).

3-Oxo-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXIX)

The hydroxy compound (CCLXXXVIII) on Jones' oxidation[71] afforded the compound (CCLXXXIX), m.p. 192°, which analysed for $C_{27}H_{42}O_5$. Its u.v. spectrum gave absorption maxima at 235 nm (ϵ8920) thus indicating the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum of compound (CCLXXXIX) showed bands at 3400-3100 (COOH), 1725s (COOH), 1700s (C=C-COOH), 1670s(C=C-C=O) and 1610 cm^{-1} (C=C).

Dimethyl 3-oxo-6,7-secocholest-4-ene-5,8-dicarboxylate (CCXC)

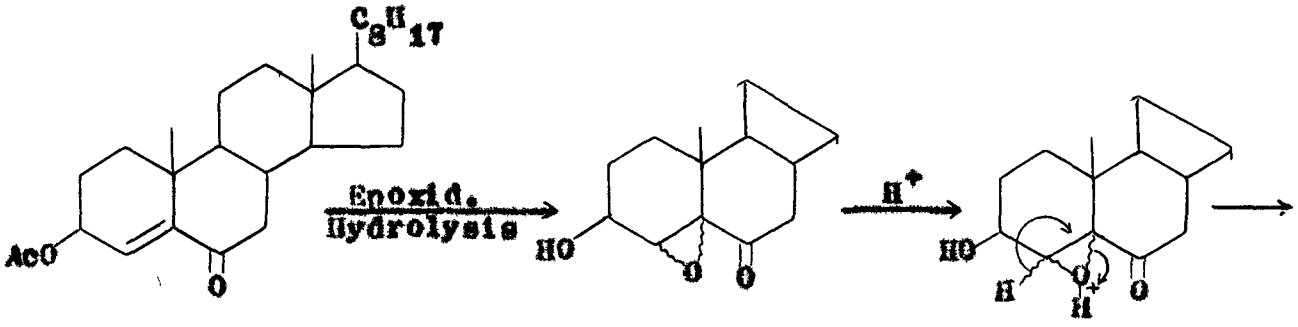
The compound (CCLXXXIX) on diazomethane treatment afforded (CCXC), m.p. 140° which analysed for $C_{29}H_{46}O_5$. Its u.v. spectrum gave absorption maxima at 236 nm (ϵ 9550), thus indicating the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum of (CCXC) showed bands at 1735s ($COOCH_3$), 1715s ($C=C-COOCH_3$), 1675s (C=C-C=O), 1620 (C=C), 1185 and 1175 cm^{-1} (methyl esters). The n.m.r. spectrum gave a singlet for 1 proton at δ 6.46 which is ascribable to C4-vinylic proton. Two sharp singlets, integrating for 3 protons each, appeared at δ 3.81 ($C5-COOCH_3$), and 3.66 ($C8-COOCH_3$). A broad multiplet spread between δ 2.9-2.4 for 3 protons is ascribable to methine and methylene protons (C8-βH and C2-H_2). Other signals were observed at δ 1.2 ($C10-CH_3$), 0.65 ($C13-CH_3$), 0.9 and 0.8 (other methyl groups).

The notable feature of the n.m.r. spectrum of (CCXC) is the downfield appearance of one of the two methoxy groups (C_5-$COOCH_3$) signal relative to (CCLXXXVII). This downfield shift is attributed to the presence of an α,β-unsaturated keto system in (CCXC)(ring A). Both the compounds (CCLXXXIX) and (CCXC) were found identical with their authentic samples prepared according to Fieser[78]. This lends further support to the structure of (CCLXXXVI).

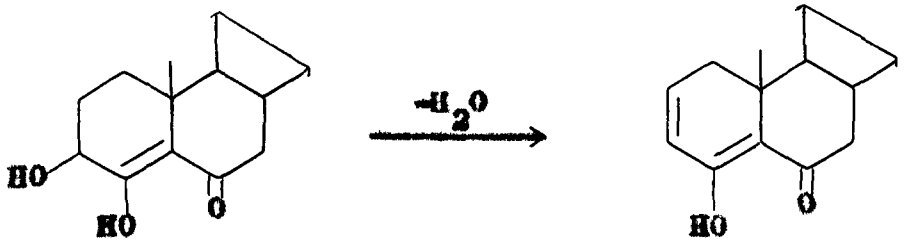
The formation of (CCLXXIV) and (CCLXXVI) from (CCLIV) may occur as shown in Scheme - 14.

Scheme - 14

a. (CCLIV) ⟶ (CCLXXIV)

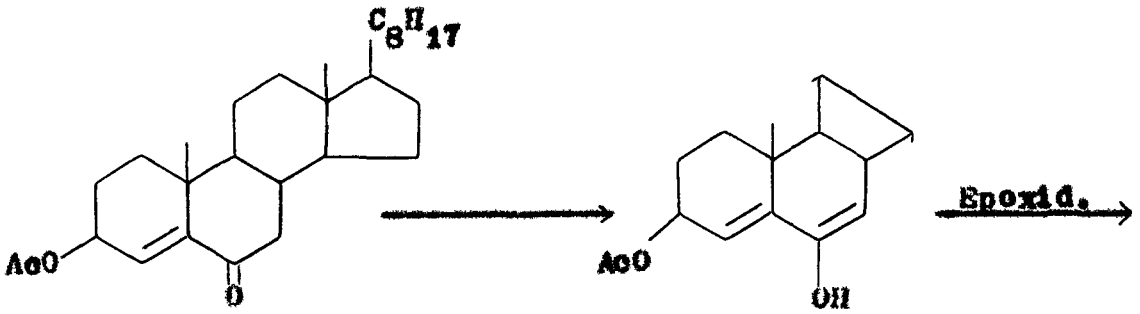


(CCLIV)

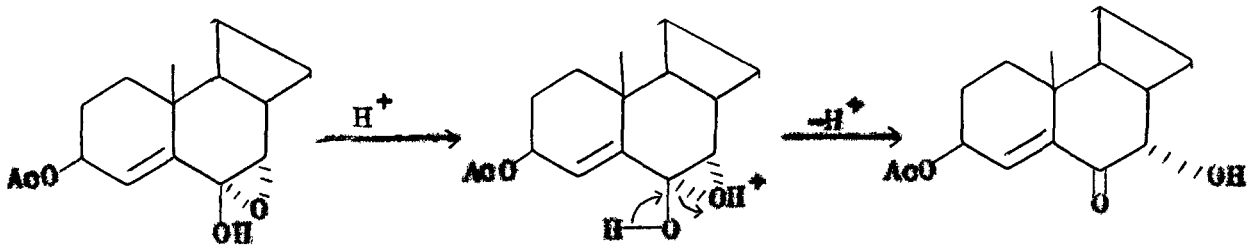


(CCLXXIV)

b. (CCLIV) ⟶ (CCLXXVI)



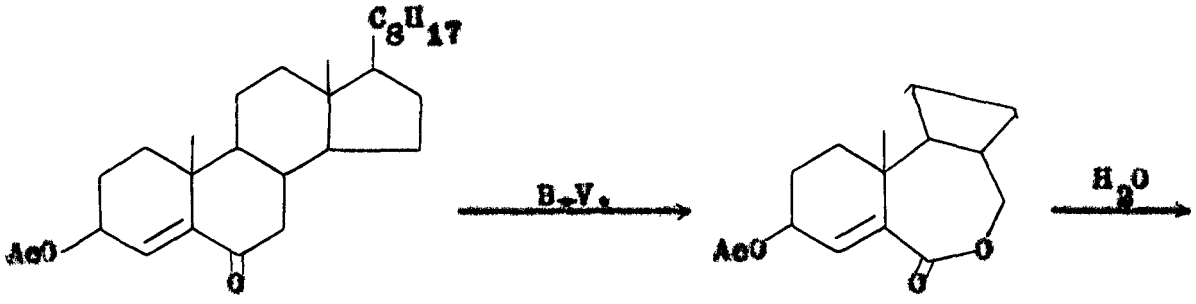
(CCLIV)



(CCLXXVI)

There are a number of possible pathways by which the formation of the secosoid (CCLXXVII) from the parent ketone (CCLIV) can be shown. One of them may involve the initial oxygen insertion between C6, C7 to give the lactone (CCXCI) in the intermediate stage which on hydrolysis and perbenzoic acid oxidation of the resultant primary alcohol function under reaction conditions, may give (CCLXXVII) (Scheme - 15).

Scheme - 16

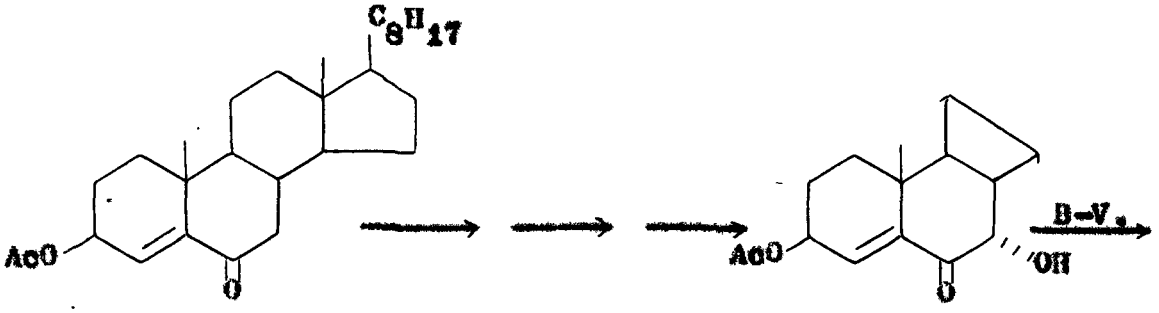


(CCLIV) (CCXCI)

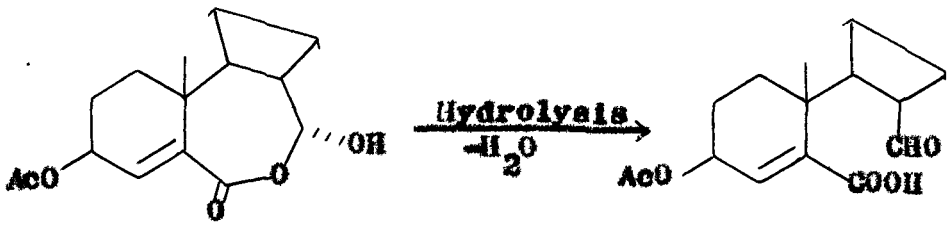
(CCLXXVII)

Another probable pathway for the formation of (CCLXXVII) from (CCLIV) may involve (CCLXXVI) as the intermediate which on oxygen insertion between C6, C7 and subsequent hydrolysis may yield (CCLXXVII) (Scheme - 16).

Scheme - 16



(CCLIV) (CCLXXVI)



(CCLXXVII)

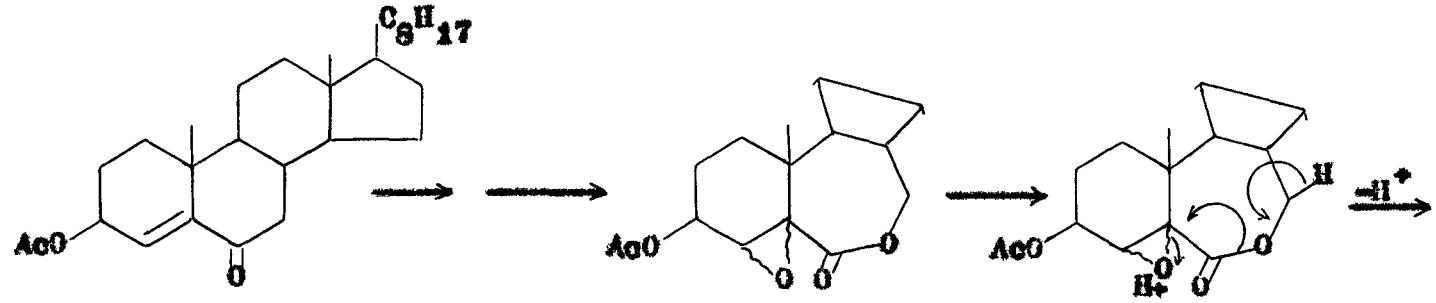
In order to check the operation of a course as in Scheme-15 in the transformation of ketone (CCLIV) to product (CCLXXVII), the aldehyde group of compound (CCLXXIX) was carefully reduced with sodium borohydride to furnish the hydroxy compound (CCLXXX) which under Baeyer-Villiger oxidation conditions did not provide the desired product (CCLXXIX); instead the unchanged starting material was recovered. Jones' oxidation of (CCLXXX) gave back the compound (CCLXXIX). Having excluded the pathway in Scheme-15, the validity of the intermediacy of (CCLXXVI) in the Scheme - 16

in the conversion of (CCLIV) ⟶ (CCLXXVII) was put to test by treating (CCLXXVI) with perbenzoic acid. This failed to provide the desired product (CCLXXVII) but gave the diacid (CCLXXXVI). Thus (CCLXXVI) is not an intermediate in the transformation (CCLIV ⟶ CCLXXVII).

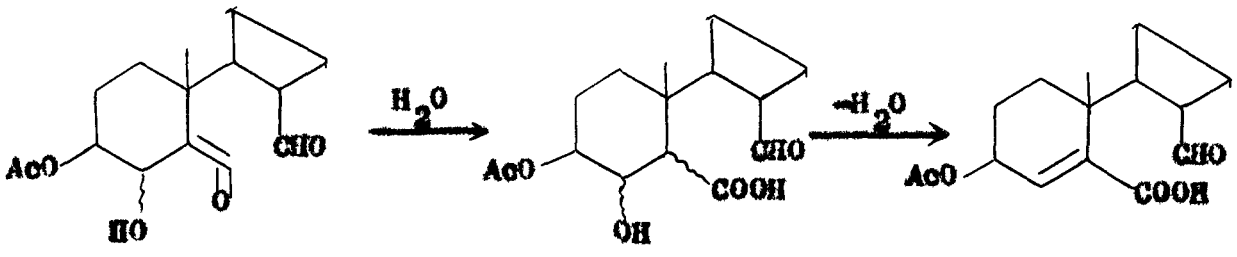
Since the formation of (CCLXXVII) requires oxygen insertion between C8, C7 and the aforesaid courses of reaction involving such an insertion are not operative, a mechanism involving initial epoxidation along with oxygen insertion between C6, C7 is being proposed (Scheme - 17).

Scheme - 17

(CCLIV) ⟶ (CCLXXVII)



(CCLIV)



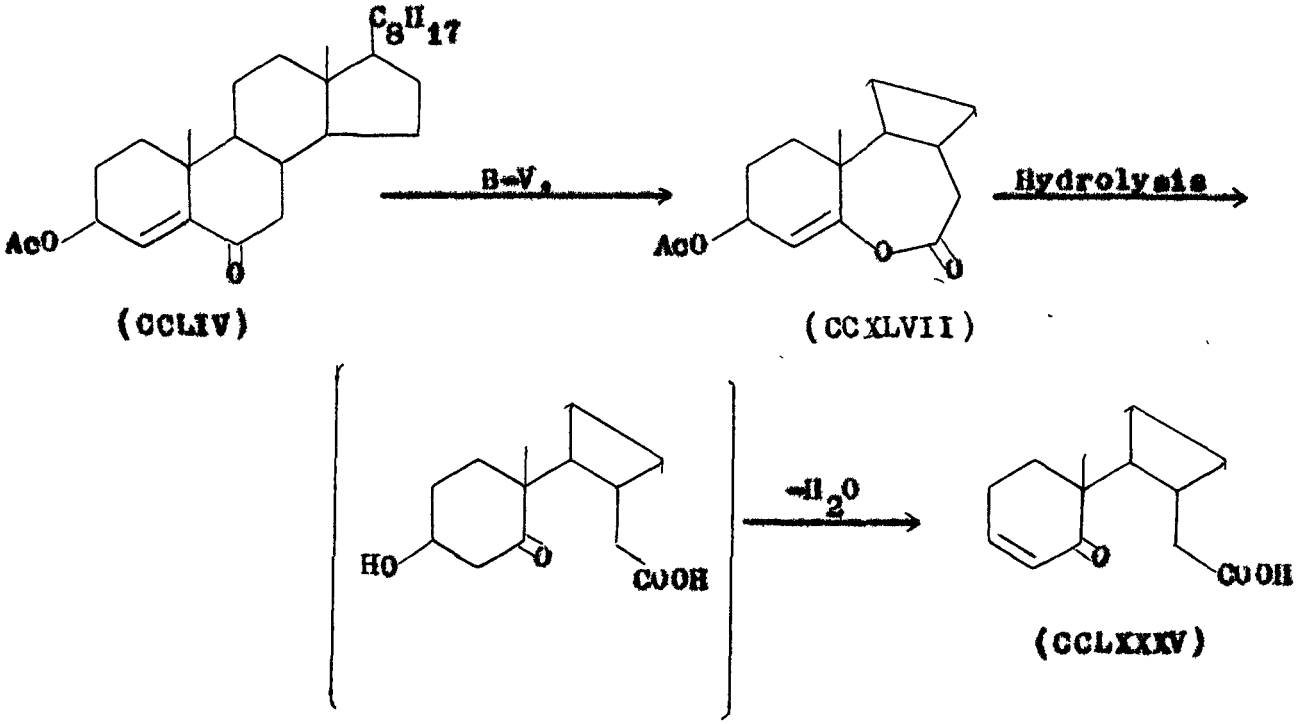
(CCLXXVII)

This peculiar cleavage of a probable lactone intermediate may well be induced by the epoxide opening which may give a ketene in the intermediate stage. Hydration of the ketene will result in an acid. Dehydration involving C4-OH is likely to be fast as it is β- to the carboxyl carbonyl. Most of the rearrangements reported in the peracid oxidation of steroidal enones proceed via epoxide intermediate[34].

The conversion of parent ketone (CCLIV) to the secoacid (CCLXXXV) has most likely involved the intermediacy of the enol lactone (CCXLVII) which could not be isolated. Hydrolyses of the enol lactone moiety, acetate function and dehydration of the subsequent β-ketol would give the secoacid (CCLXXXV) (Scheme - 18). This suggestion finds support from an earlier observation[79] when (CCXLVII) on methanolysis was found to give (CX).

Scheme - 18

(CCLIV) ⟶ (CCLXXXV)



(CCLIV) (CCXLVII) (CCLXXXV)

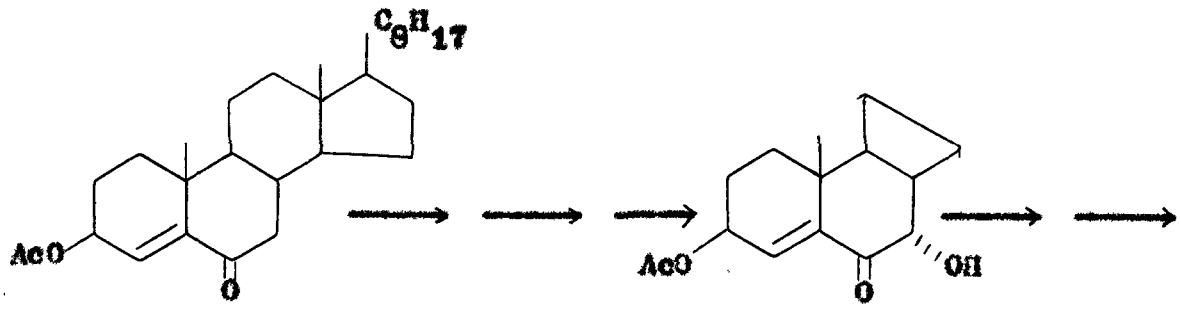
From the close structural relationship between 7-oxo-secocacid (CCLXXVII) and the diacid (CCLXXXVI), it was suspected that (CCLXXVII) may be the precursor of (CCLXXXVI) in the sense that the former is oxidised in the presence of perbenzoic acid. In view of the previous observation that a primary alcohol like (CCLXXX) is not oxidised to (CCLXXIX) by perbenzoic acid, the oxidation of (CCLXXVII) to (CCLXXXV) under the Baeyer-Villiger oxidation reaction conditions seemed somewhat unlikely. This was substantiated when (CCLXXVII) on treatment with perbenzoic acid did not afford (CCLXXXVI). The C7-oxo group in (CCLXXVII) remained unaffected even with Jones' oxidation.

It has already been pointed out that (CCLXXVI) furnishes the desired compound (CCLXXXVI) under the Baeyer-Villiger oxidation reaction conditions. This fact demands a mechanism which should involve (CCLXXVI) as one of the intermediates in the formation of (CCLXXXVI) from the starting ketone (CCLIV). Several possible mechanisms which involve (CCLXXVI) as an intermediate are being proposed in Scheme - 19. One of the mechanisms (mechanism a) is analogous with that described for the conversion (CCLIV) ⟶ (CCLXXVII) in that it also involves an epoxide and its subsequent role in directing the lactone opening.

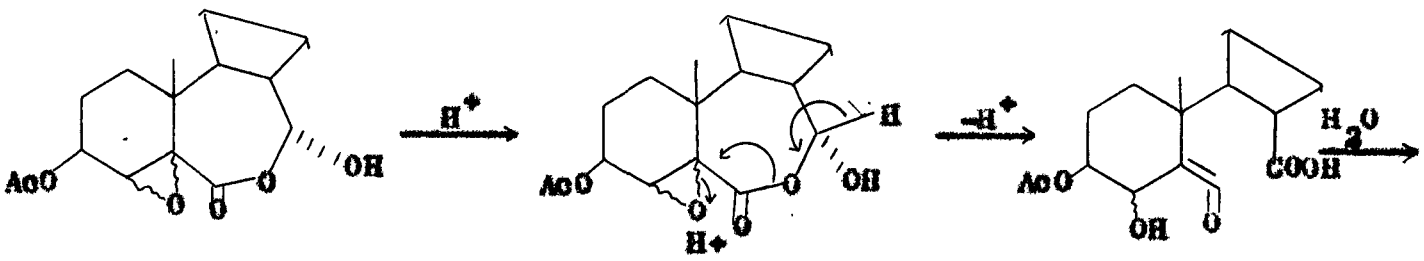
Scheme - 19

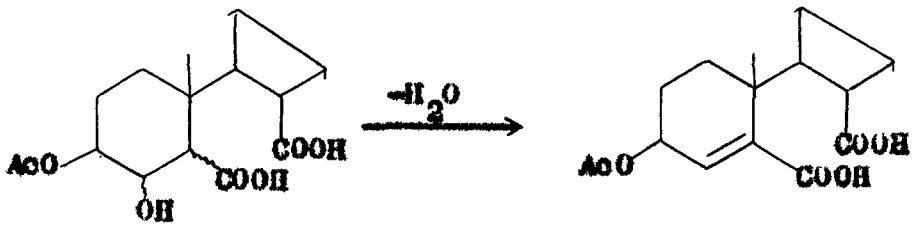
(CCLIV) ⟶ (CCLXXXVI)

Mechanism



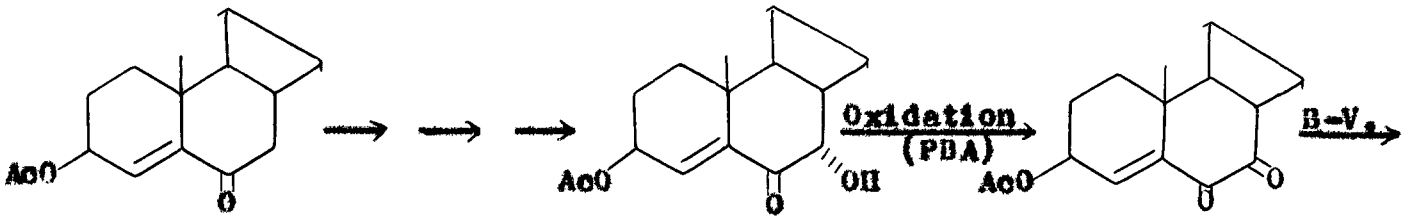
(CCLIV) (CCLXXVI)



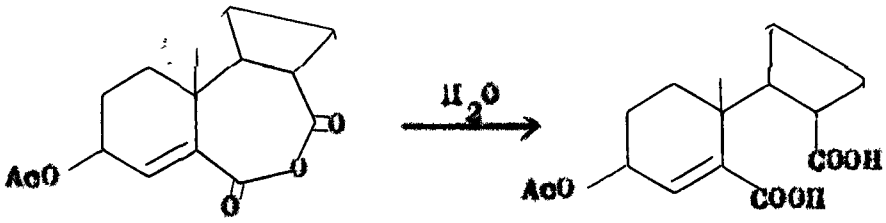


(CCLXXXVI)

Mechanism-b

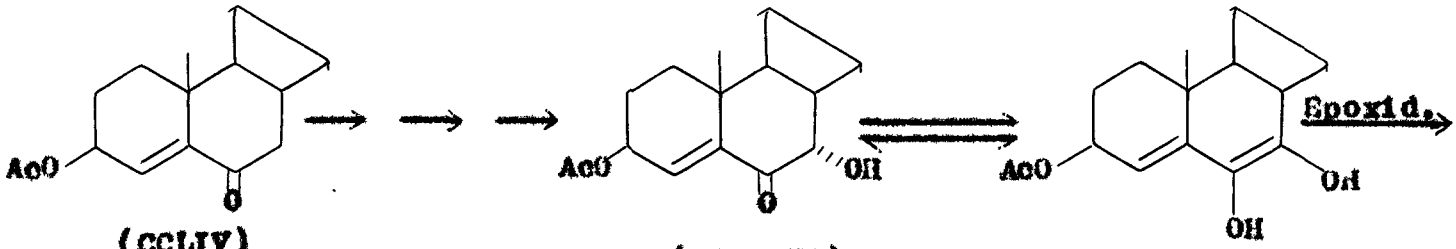


(CCLIV) (CCLXXVI)(α-ketol)

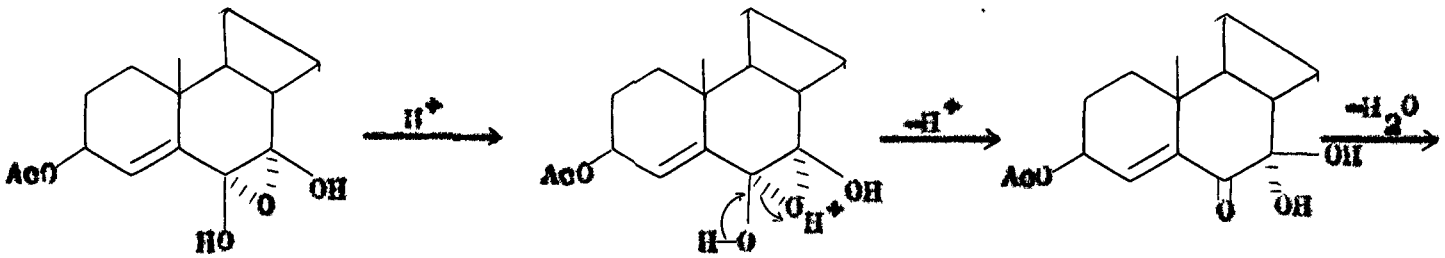


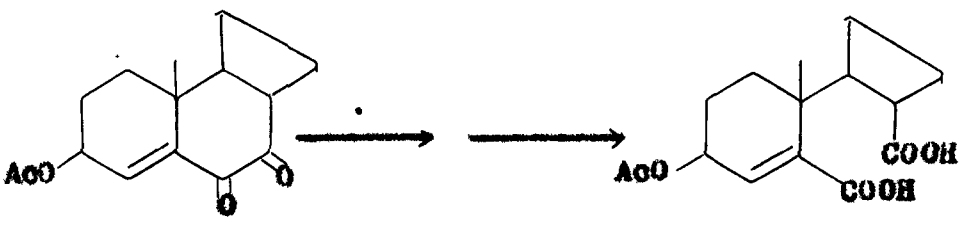
(CCLXXXVI)

Mechanism-c



(CCLIV) (CCLXXVI)





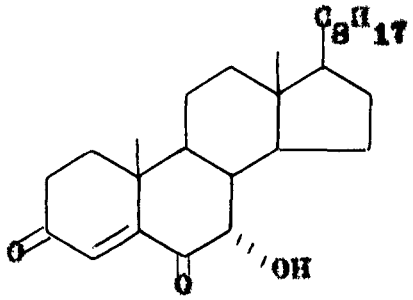
(CCLXXXVI)

Reaction of cholest-4-ene-3,6-dione (CCXXI) with perbenzoic acid

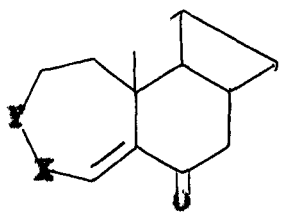
Reaction of cholest-4-ene-3,6-dione (CCXXI) with perbenzoic acid gave a variety of products depending upon the quantity of the peracid used. With one mole equivalent of perbenzoic acid, (para-toluenesulphonic acid monohydrate as catalyst) the ketone (CCXXI) gave three compounds, m.pts. 100°, 165° and 195°. Reaction of (CCXXI) with two mole equivalent of perbenzoic acid afforded another compound, m.p. 115° along with the compound, m.p. 195°. With three mole equivalent of perbenzoic acid (CCXXI) gave the compound, m.p. 115° along with another compound, m.p. 193°.

Characterization of the compound, m.p. 165° as 7α-hydroxycholest-4-ene-3,6-dione (CCXCII)

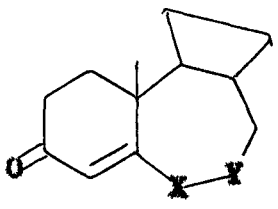
The compound, m.p. 165° analysed for $C_{27}H_{42}O_3$ which indicated the incorporation of only one oxygen atom to the parent ketone (CCXXI). This composition leads to several possibilities such as (CCXCII-CCXCIIe).



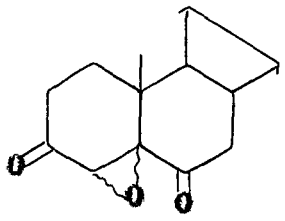
(CCXCII)



(CCXCIIa) X,O; Y,CO
(CCXCIIc) X,CO; Y,O



(CCXCIIb) X,O; Y,CO
(CCXCIId) X,CO; Y,O



(CCXCIIe)

The u.v. spectrum of the compound showed absorption maxima at 245 nm (ϵ 12216), thus showing the presence of an α,β-unsaturated carbonyl chromophore as in (CCXCIIa-CCXCIId) but excluded the epoxide (CCXCIIe). The i.r. spectrum gave bands at 3330br (OH), 1700s (HO - C - C=O, α-hydroxycarbonyl), 1680s (C=C-C=O), 1625 (C=C), 1225 and 1040 cm^{-1}(C-O). These values are not compatible with the presence of enol lactone carbonyl as in (CCXCIIa) or (CCXCIIb) where one would have expected much higher values for such carbonyl grouping. These values also excluded (CCXCIIe). The presence of an OH group in the compound supported the structure (CCXCII). The n.m.r. spectrum gave a sharp singlet at δ 6.1 integrating for 1 proton which can be ascribed to a vinylic proton. An unresolved singlet like signal appeared at δ 3.98 integrating for 1 proton which has been assigned to C7-βH (equatorial) in (CCXCII). An examination of the Dreiding model of (CCXCII) revealed that the dihedral angle between C7-βH and C8-βH is almost 90° and therefore C7-βH appeared as an unresolved signal. The hydroxyl proton of (CCXCII) was merged with methylene protons (CH_2 - C(=O)-) at δ 2.3 as revealed by deuterium exchange.

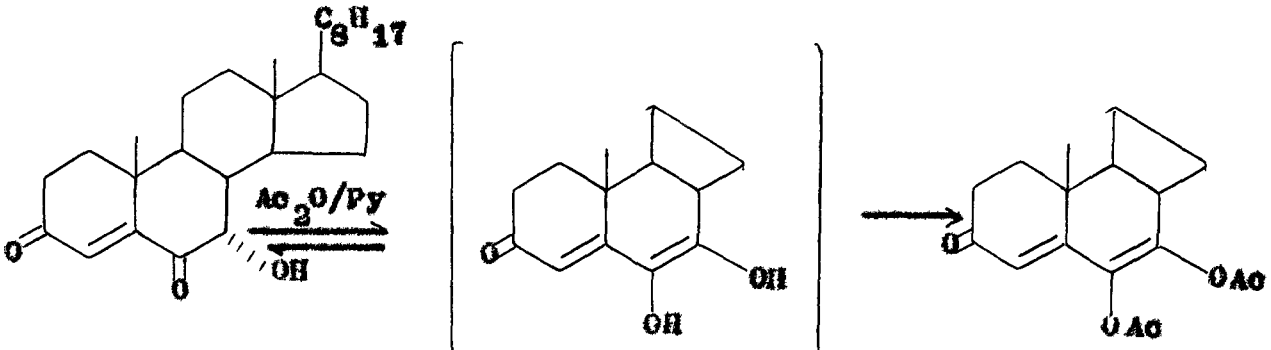
After D_2O shake, a multiplet at δ 2.3 was observed for 2 protons which is ascribable to methylene protons ($-C\underline{H}_2 - \overset{O}{\overset{\|}{C}}-$). Other signals were seen at δ 1.15 (C10-CH_3), 0.7 (C13-CH_3), 0.9 and 0.81 (other methyl groups). On the basis of spectral properties the compound m.p. 165° has been assigned the structure (CCXCII). A possible mechanism for the conversion of (CCXXI) to (CCXCII) can be proposed in an analogous manner as shown in Scheme-14b.

6,7-Diacetoxycholesta-4,6-dien-3-one (CCXCIII)

To substantiate the presence of an hydroxy group in the compound (CCXCII), it was heated with acetic anhydride-pyridine which afforded the diacetate (CCXCIII), m.p. 125° and analysed for $C_{31}H_{46}O_5$. Its u.v. spectrum[74] showed absorption maxima at 320 nm (ϵ 21704), thus showing the presence of a conjugated dienone chromophore. The i.r. spectrum[75] gave bands at 1760s, 1750s (2 enolacetate carbonyls), 1680s (C=C-C=C-C=O), 1630 (C=C-O), 1570 (C=C-C=C-), 1220, 1210 (acetate) and 1020 cm^{-1} (C-O). The n.m.r. spectrum of the diacetate (CCXCIII) gave a signal at δ 6.11 as a doublet (J 1.5 Hz) integrating for 1 proton which can be ascribed to C4-vinylic proton having a long range coupling with C2-H (M-pattern). A multiplet at δ 2.35 for 2 protons was assigned to methylene protons ($-C2\underline{H}_2-\overset{O}{\overset{\|}{C}}-$). Two sharp singlets at δ 2.2 and 2.15, each integrating for 3 protons are ascribable to two acetate methyl groups. Other signals were seen at δ 1.2 (C10-CH_3), 0.7 (C13-CH_3),

0.9 and 0.8 (other methyl groups).

Formation of the diacetate (CCXCIII) is possible only through enolization of parent compound (CCXCII) under the reaction conditions and its subsequent acylation to give the diacetate.



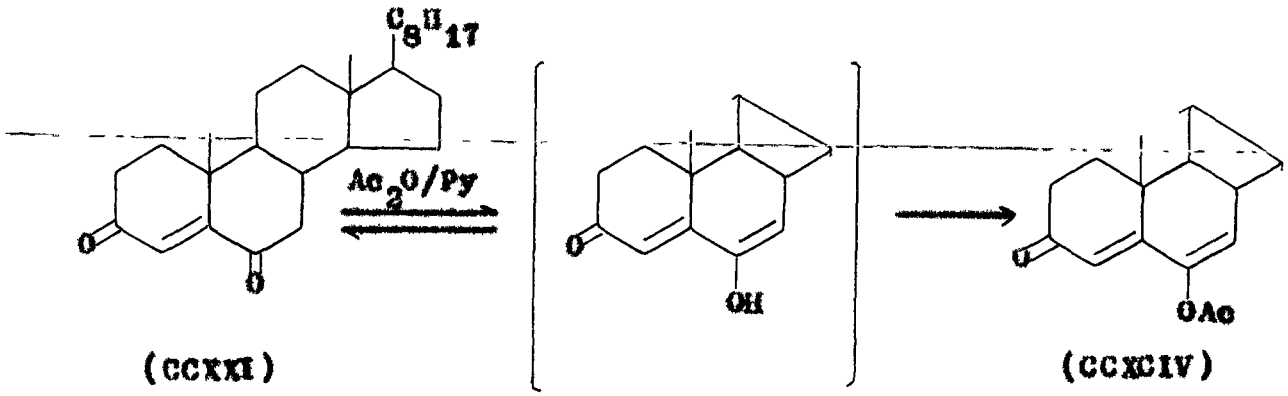
(CCXCII) (CCXCIII)

Attempts to prepare a monoacetate from (CCXCII) by employing milder conditions (acetic anhydride-pyridine, room temperature) invariably resulted in the formation of the diacetate (CCXCIII).

6-Acetoxycholesta-4,6-dien-3-one (CCXCIV)

The formation of a diacetate (CCXCIII) from (CCXCII) demands that the latter undergoes enolization during acetylation. For further support, the starting ketone (CCXXI) was heated with acetic anhydride-pyridine and this also afforded the corresponding enol acetate (CCXCIV), m.p. 112°, which analysed for $C_{29}H_{44}O_3$. The u.v. spectrum[74] of (CCXCIV) showed absorption maxima at 298 nm (ϵ 23200)

thus indicating the presence of a conjugated dienone chromophore. Its i.r. spectrum[75] gave bands at 1770s (enolacetate carbonyl), 1680s (C=C-C=C-C=O), 1640 (C=C-O), 1590 (C=C-C=C), 1210 and 1190 cm^{-1} (acetate). The n.m.r. spectrum of the compound, m.p. 112° gave signals at δ 5.78d (1H; J 1.5 Hz) and 5.75 (1H; singlet like) which are ascribable to two vinylic protons C4-H and C7-H (unassigned). The Dreiding model of (CCXCIV) showed the dihedral angle between C7-H and C8-βH to be about 90°. A multiplet at δ2.4 integrating for 2 protons is ascribable to methylene protons (C2-H_2). A sharp singlet at δ 2.18 was assigned to methyl group of acetate function (CH_3COO). Other signals were seen at δ 1.18 (C10-CH_3), 0.76 (C13-CH_3), 0.9, 0.81 (other methyl groups).



(CCXXI) (CCXCIV)

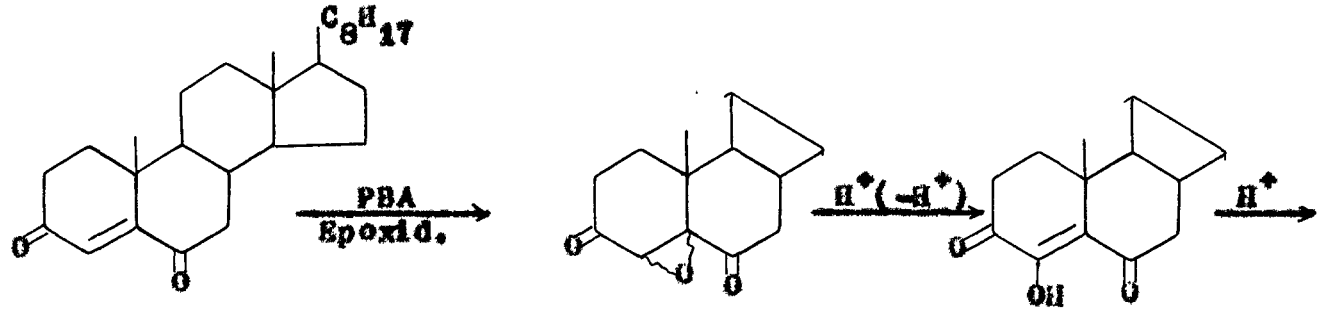
Characterisation of the comnound, m.p. 100° as 4-hydroxy-6-methoxycholesta-4,6-dien-3-one (CCXCV)

The compound, m.p. 100° analysed for $C_{28}H_{44}O_3$ and it was characterized as (CCXCV) on the basis of elemental analysis ($C_{29}H_{44}O_3$) and spectral properties. Its u.v. spectrum[74] showed absorption maxima at 335 nm (ϵ 22700) thus indicating the presence of a conjugated dienone chromophore. The i.r. spectrum[75] gave bands at 3400br (OH), 1650s (C=C-C=C-C=O), 1603 (C=C-C=C), 1200, 1060 and 1030 cm^{-1} (C-O). The n.m.r. spectrum of (CCXCV) gave a signal at δ 5.76 as doublet (J 1 Hz) for 1 proton, which is easily ascribable to vinylic proton (C7-H). An examination of the Dreiding model of (CCXCV) once again revealed that the dihedral angle between C7-H and C8-βH is almost 90° and therefore the signal is only partially split. A sharp singlet for 3 protons was observed at δ 3.71 which clearly indicated the presence of a methoxy group ($-OCH_3$). The hydroxy proton appeared along with the methylene protons at δ 2.3 ($C2-H_2$) and this was revealed by deuterium exchange which changed the pattern of the signal at δ 2.3. Other signals were observed at δ 1.1 ($C10-CH_3$), 0.73 ($C13-CH_3$), 0.91 and 0.81 (other methyl groups).

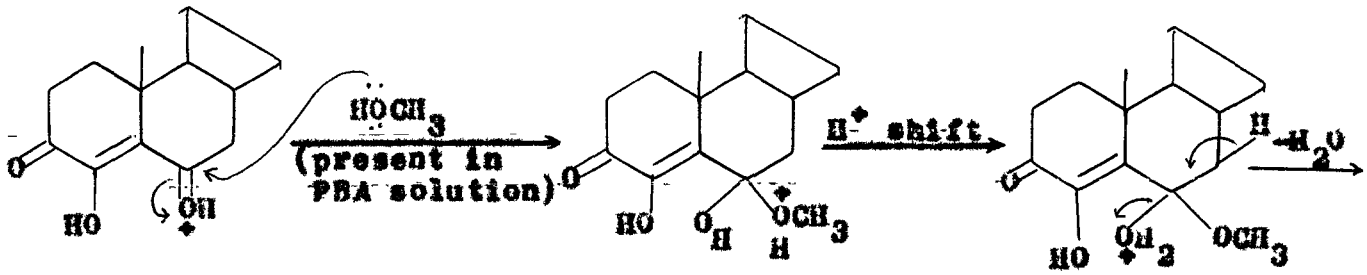
A possible mechanism for the conversion of (CCXXI) to (CCXCV) is given in Scheme - 20.

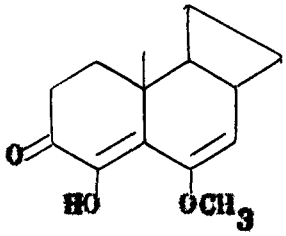
Scheme - 20

(CCXXI) ⟶ (CCXCV)



(CCXXI)





(CCXCV)

Tentative characterisation of the compound, m.p. 195° as 3-hydroxy-3,4-oxidocholest-4-en-6-one (CCXCVI)

The compound, m.p. 195°, analysed for $C_{27}H_{44}O_3$ and was indicated to be an isomer of (CCXCII). This composition can lead to several possibilities as discussed in connection with (CCXCII). The u.v. spectrum of (CCXCVI) showed absorption maxima at 275 nm (ϵ 6575) thus suggesting the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum displayed bands at 3420br (OH), 1670s (C=C-C=O), 1620 (C=C) and 1060 cm^{-1} (C-O). Its n.m.r. spectrum gave no signal for a vinylic proton. An unresolved singlet like signal was observed at δ 3.08 integrating for 1 proton. The hydroxyl proton merged with methylene protons at δ 2.3 as revealed by deuterium exchange which affected the pattern of this signal. Other signals were seen at δ 1.2 (C_{10}-CH_3), 0.66 (C_{13}-CH_3), 0.9 and 0.81 (other methyl groups). Though these values are compatible with the structure (CCXCVI), we make this assignment with some reservation.

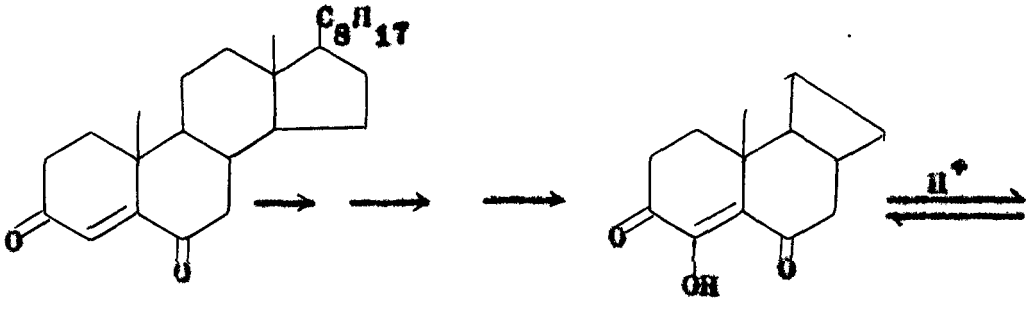
3-Acetoxy-3,4-oxidocholest-4-en-6-one (CCXCVII)

The hydroxy compound (CCXCVI) was converted to its acetate (CCXCVII), m.p. 165°, which analysed for $C_{29}H_{44}O_4$. The u.v. spectrum showed absorption maxima at 268 nm (ϵ 8491) thus indicating the presence of an α,β-unsaturated carbonyl chromophore. The i.r. spectrum gave bands at 1740s (CH_3COO), 1690s (C=C-C=O), 1642 (C=C),

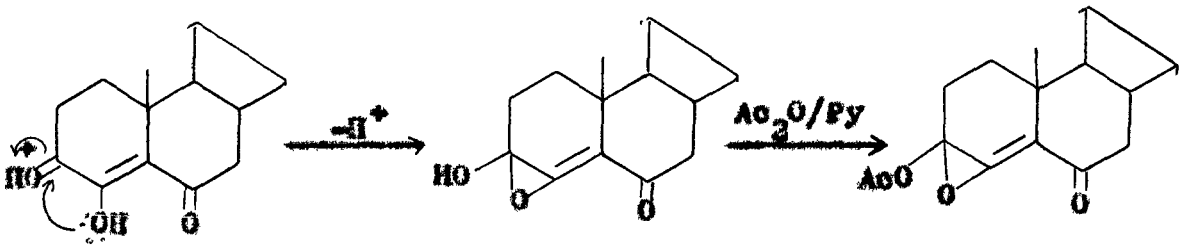
1240 (acetate), 1150, 1120 and 1020 cm^{-1}(C-O). The band at 1740 cm^{-1} discarded the possible presence of an enolic acetate function. Further, the n.m.r. spectrum of compound, m.p. 145° gave no signal for a vinylic proton but an unresolved singlet like signal for 1 proton appeared at δ 3.66. A multiplet for methylene protons appeared at δ 2.4. A sharp singlet at δ 2.0 integrating for 3 protons, could be ascribed to acetate methyl protons ($C\underline{H}_3COO$). Other signals were seen at δ 1.20 ($C10-CH_3$), 0.66 ($C13-CH_3$), 0.9 and 0.81 (other methyl groups).

The signals at δ 3.08 and 3.66 in (CCXCVI) and (CCXCVII), respectively, are difficult to interpret. However, it is reasonable to assign these signals to one of the two protons at C2, i.e. $\underline{H}-C_2-C_3$(OH, O) , $\underline{H}-C2-C3$(OAc, O) groupings present in (CCXCVI) and (CCXCVII) respectively. The singlet like nature of these signals could be due to some deformity in ring A caused by the peculiar assembly of functional groups in the ring. On the basis of spectral properties, compound, m.p. 195° and its acetate m.p. 145° have been assigned the structures (CCXCVI) and (CCXCVII), respectively. A possible pathway for the conversion of (CCXXI) to (CCXCVI) and its acetylation to (CCXCVII) is shown in Scheme - 21.

Scheme - 21



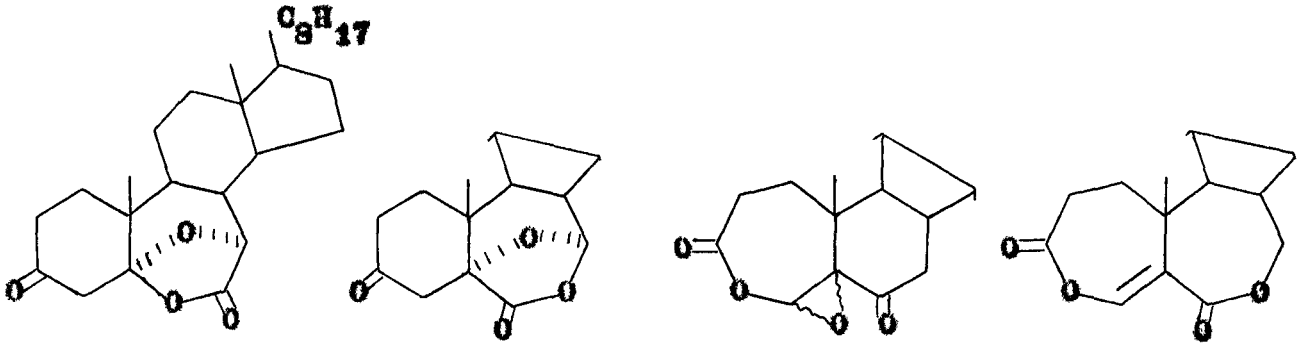
(CCXXI)



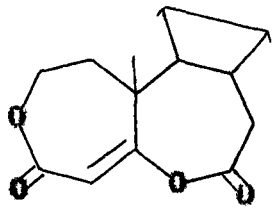
(CCXCVI) (CCXCVII)

Characterization of the compound, m.p. 115° as 5∝,7a∝-oxido-6-oxa-B-homocholestane-3,7-dione (CCXCVIII)

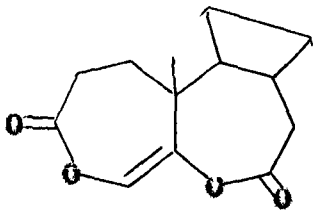
The compound, m.p. 115° analysed for $C_{27}H_{42}O_4$ and this composition was supported by its mass spectrum which gave molecular ion peak at m/e 430 ($C_{27}H_{46}O_4$). From the composition it is evident that two atoms of oxygen have been added during the course of reaction and this leads to several possibilities (CCXCVIII, CCXCVIIIa, CCXCIX-CCCIII). The i.r. spectrum[75] gave bands at 1798s, 1720s, 1180m, 1140m and 920s cm^{-1}. The u.v spectrum was



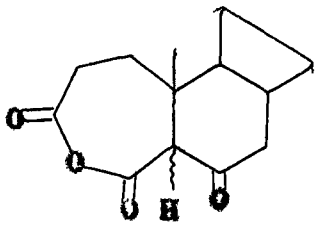
(CCXCVIII) (CCXCVIIIa) (CCXCIX) (CCC)



(CCCI)



(CCCII)



(CCCIII)

found to be featureless in the region 200–360 nm, thus indicating the absence of an α,β-unsaturated carbonyl chromophore in the molecule. The n.m.r. spectrum gave signals at δ 5.6 (1H), 2.9d (1H, J 15 Hz; gem coupling) and 2.3d (1H, J 15 Hz; gem coupling). The latter two signals are ascribable to 2 nonequivalent protons of an isolated methylene group (AB system centred at δ 2.6)[80]. Other signals were seen at δ 1.01 ($C10\text{-}CH_3$), 0.70 ($C13\text{-}CH_3$), 0.9 and 0.8 (other methyl groups). Besides (CCXCVIII), several other structures such as (CCXCVIIIa, CCXCIX-CCCIII) with the composition $C_{27}H_{42}O_4$ can be formulated for this product, which are logically derivable from (CCXXI) under the reaction conditions.

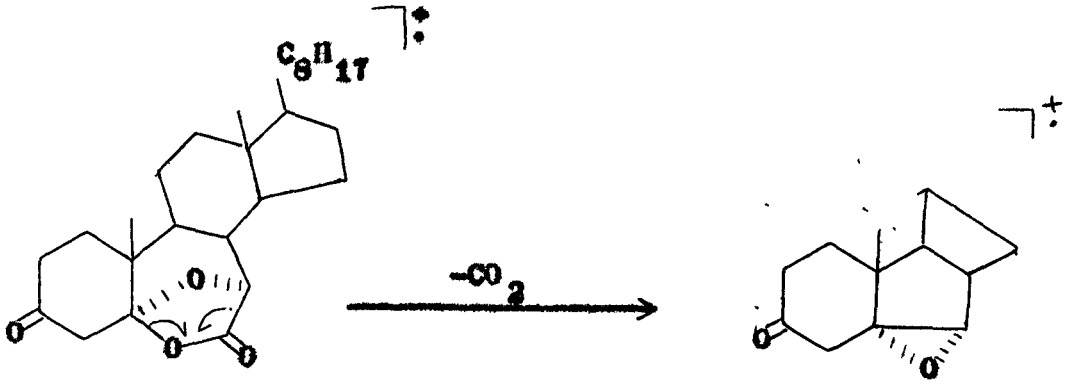
On the basis of spectral properties, most of the structures (CCXCIX-CCCIII) can be discarded, since none of them possess an isolated methylene group adjacent to a carbonyl function as demanded by the n.m.r. spectrum (2.9d and 2.3d). Structures (CCC-CCCII) show the presence of a carbon-carbon double bond with one vinylic proton but the i.r. spectrum of the compound gave no indication of the presence of such a moiety. Further a strong band at

1798 cm^{-1} cannot be reconciled with the structures (CCXCIX-CCCII). Strong bands at about 1800 and 1740 cm^{-1} are usually observed for cyclic anhydrides[75] such as in (CCCIII), but this structure also lacks the presence of an isolated methylene group. With the elimination of the structures (CCXCIX-CCCIII) the choice of the structures narrowed down to (CCXCVIII) and (CCXCVIIIa).

The n.m.r. signal at δ 5.6 may be confused with a vinylic proton. An examination of the Dreiding model of (CCXCVIII) showed that the dihedral angle between C7a-H and C8-βH is almost 90° and this led to the assignment of signal at δ 5.6 to C7a-H in (CCXCVIII). On the other hand the model of (CCXCVIIIa) showed that there should be small splitting of C7a-H.

The mass spectrum of (CCXCVIII) gave molecular ion peak at m/e 430 with other peaks in the higher mass region at m/e 415 ($M-CH_3$), 412 ($M-H_2O$), 402 (M-CO) and 386 ($M-CO_2$; base peak). The loss of CO_2 from the molecular ion is compatible with the assigned structure (CCXCVIII) and can be shown to occur according to Scheme - 22.

Scheme - 22

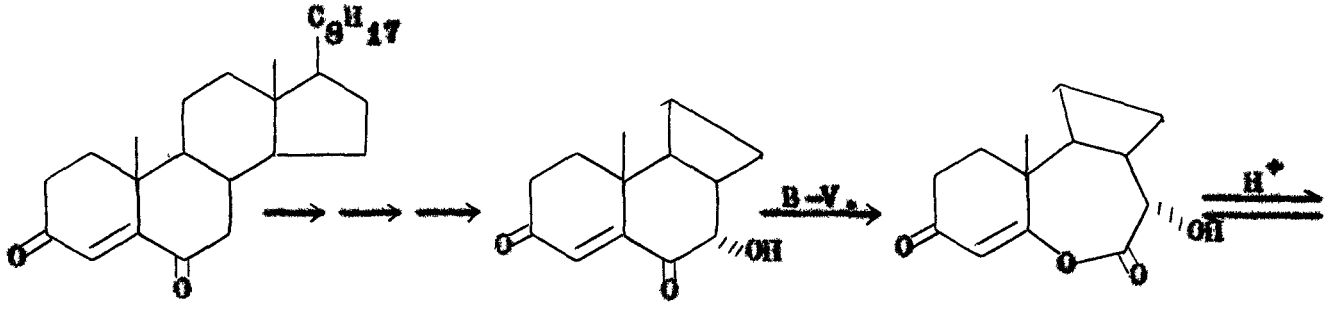


(CCXCVIII') m/e 386

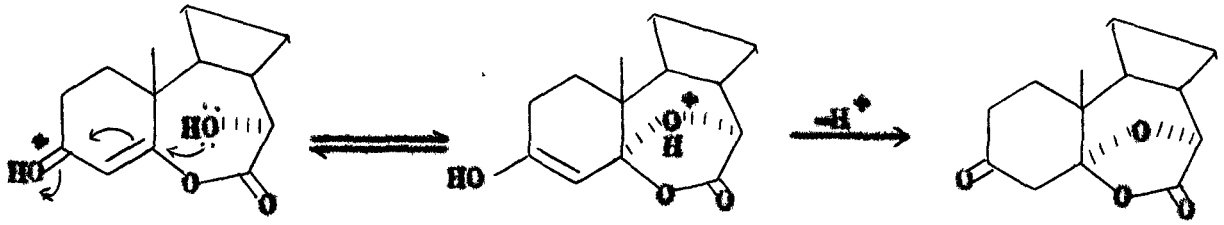
When the hydroxy compound (CCXCII) was subjected to perbenzoic acid (1 mole equivalent) oxidation, it afforded (CCXCVIII). It therefore appears to be an intermediate in the transformation of (CCXXI) to (CCXCVIII). A possible mechanism for the formation of isomeric products (CCXCVIII) and (CCXCVIIIa) from (CCXXI) under Baeyer-Villiger oxidation conditions involving the intermediacy of (CCXCII) can be proposed as in Scheme - 23.

Scheme - 23

A. (CCXXI) ⟶ (CCXCVIII)

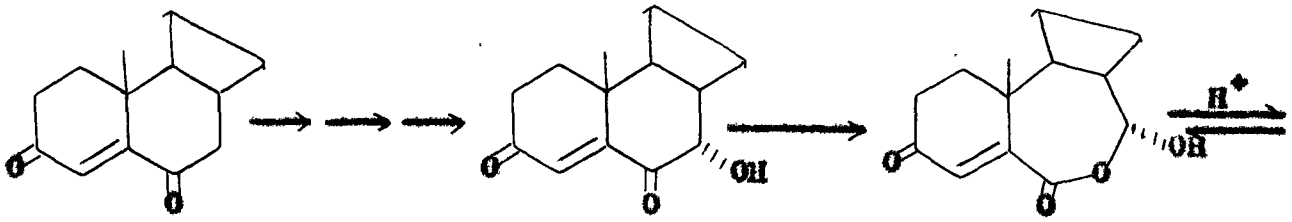


(CCXXI) (CCXCII)

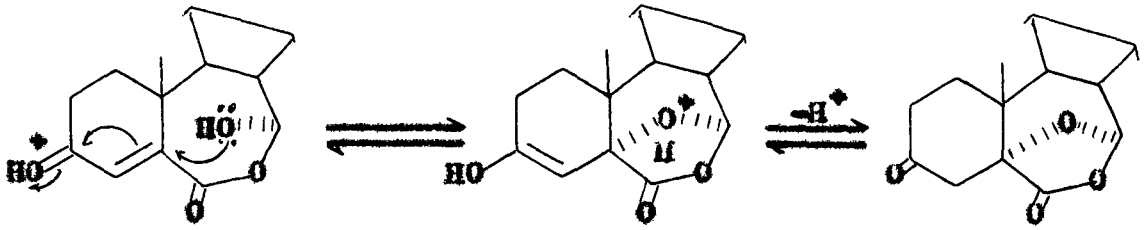


(CCXCVIII)

8. (CCXXI) ⟶ (CCXCVIIIa)



(CCXXI) (CCXC)



(CCXCVIIIa)

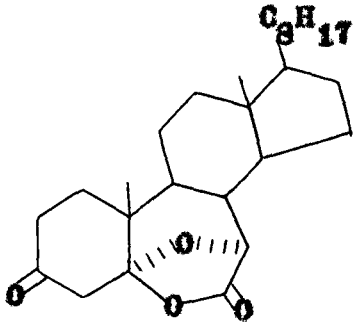
From the mechanistic and over all spectral considerations, the compound, m.p. 115° has been assigned the oxetalactone structure (CCXCVIII). It is reasonable to suggest that the migration of a vinylic carbon (C5) may occur in preference to C7 to give (CCXCVIII). The strong bands in the i.r. spectrum of (CCXCVIII) at 1798 and 920 cm^{-1} can be ascribed to C=O and C-O-C linkage of the oxeta lactone moiety.

Characterization of the compound, m.p. 193° as 5α,7aα-oxido-3,6-dioxa-A,B-bishomocholestane-4,7-dione (CCCIV)

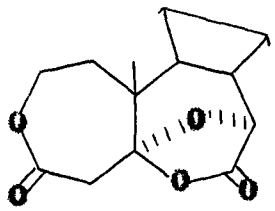
The compound, m.p. 193° analysed for $C_{27}H_{42}O_5$ and this composition was supported by its mass spectrum (M^+ 446; $C_{27}H_{42}O_5$). The composition suggested the addition of three atoms of oxygen to the starting ketone (CCXXI). The i.r. spectrum[75] of the compound m.p. 193° showed bands at 1795s, 1735s, 1200m, 1142m and 920s cm^{-1}, and its u.v. spectrum like (CCXCVIII), was found featureless in the region 200-360 nm. In these respects, this compound resembled strongly with (CCXCVIII). The strong bands at 1795 and 920 cm^{-1} suggested the presence of oxetalactone moiety in this compound also. The n.m.r. spectrum of the compound gave signals at δ 5.6s (1H), 4.4 (2H, $\underline{H}_2C_2$-OCO), 3.2d (1H, J 15 Hz; gem coupling) and 2.6d (1H, J 15 Hz; gem coupling); the latter two signals are ascribable to the two nonequivalent protons of an isolated methylene group (AB system centred at δ 2.9)[80]. Other signals were seen at δ 1.05 (C10-CH_3), 0.7 (C13-CH_3), 0.9 and 0.8 (other methyl groups). The mass spectrum of the compound, m.p. 193° gave molecular ion peak at m/e 446 ($C_{27}H_{42}O_5$) with other peaks in the high mass region at m/e 431 (M-CH_3), 428 (M-H_2O), 418 (M-CO) and 402 (M-CO_2).

The close similarity between the spectral properties of the compounds, m.p. 193° and (CCXCVIII) suggested strong structural resemblance between the two. Evidently there is a difference of only one oxygen atom between (CCXCVIII) and compound, m.p. 193°

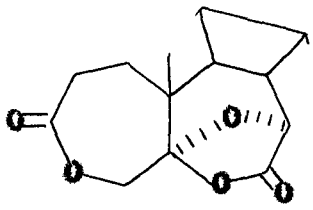
($C_{27}H_{42}O_5$) and it was suspected that insertion of an oxygen atom to (CCXCVIII) has taken place during the reaction. In chemical support of this proposal, (CCXCVIII) was subjected to Baeyer-Villiger oxidation which afforded (CCCIV).



(CCXCVIII)



(CCCIV)

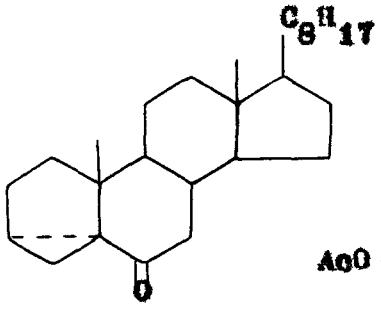


(CCCIVa)

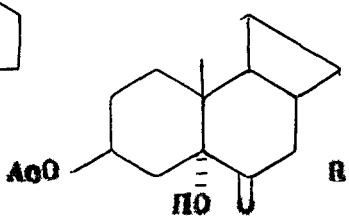
Insertion of one oxygen to (CCXCVIII) may lead to (CCCIV) and its isomer (CCCIVa). The structure (CCCIVa) has been discarded, since in this case the methylene group, C_2H_2-COO- in n.m.r. spectrum would have given a multiplet at about δ 2.4. The slight downfield shift of the isolated methylene protons signals in the compound (CCCIV) is understandable since it has an additional oxygen in close vicinity.

Steroidal Tetrazoles

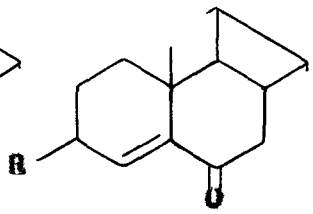
In the recent past, several steroidal tetrazoles were synthesized in our laboratory by reaction of steroidal ketones with an excess of hydrazoic acid and these included 3α,5-cyclo-5α-cholestan-6-one (CCX), 3β-acetoxy-5-hydroxy-5α-cholestan-6-one (CCXIII)[54], cholest-4-en-6-one (CCCV) and its 3β-acetoxy derivative (CCLIV), 5α-cholestane-3,6-dione (CCXV) and its 5α-hydroxy derivative (CCXVIII), cholest-4-ene-3,6-dione (CCXXI), 6β-bromo-cholest-4-en-3-one (LXXXIII)[55], cholest-5-en-7-one (XCI), its 3β-chloro (CCXXV) and acetoxy (LXXXVI) analogues, and cholesta-3,5-dien-7-one (CCXXX)[54].



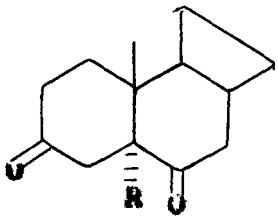
(CCX)



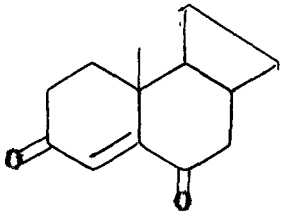
(CCXIII)



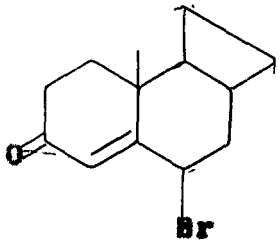
(CCCV) R,H
(CCLIV) H,OAc



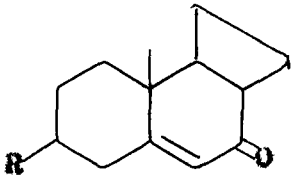
(CCXV) R,H
(CCXVIII) R,OH



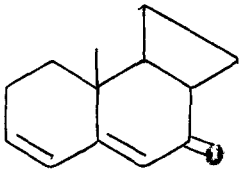
(CCXXI)



(LXXXIII)

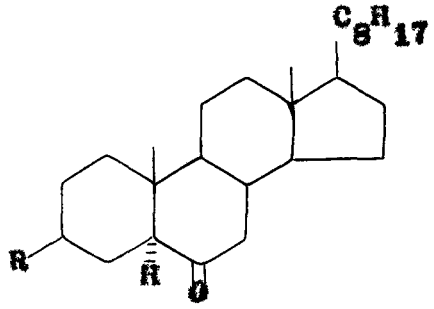


(XCI) R,H
(CCXXV) R,Cl
(LXXXVI) R,OAc



(CCXXX)

The present work describes the preparation of tetrazoles derived from hitherto unexplored steroidal 6-ones such as 5α-cholestan-6-one (XXXIII) and its 3β-acetoxy (XXXIV), 3β-hydroxy (CCLIII) and 3β-chloro (CCXLIX) analogues.



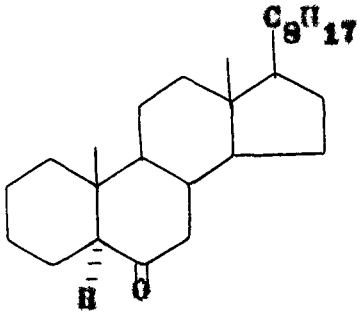
(XXXIII) R,H
(XXXIV) R, OAc
(CCLIII) R, OH
(CCXLIX) R, Cl

Reaction of 5α-cholestan-6-one (XXXIII) with an excess of hydrazoic acid

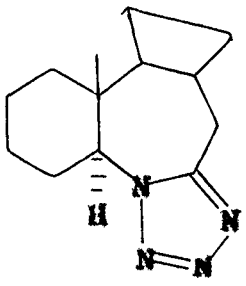
5α-Cholestan-6-one (XXXIII) was treated with an excess of hydrazoic acid solution (prepared according to the method described by Moural and Syhora[45]) in the presence of borontrifluoride etherate as catalyst. Usual work up of the reaction mixture and column chromatography over silica gel provided two products, m.pts. 169° and 175°.

Characterization of the compound, m.p. 169° as 6-aza-B-homo-5∝-cholestano[6,7-d]tetrazole (CCCVI)

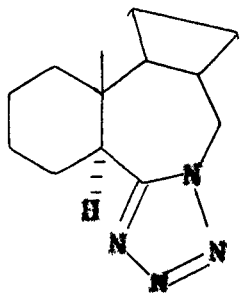
The compound, m.p. 169° analysed for $C_{27}H_{46}N_4$ and the formation of a tetrazole was evident from its elemental analysis. Its i.r. spectrum exhibited peaks at 1540, 1460 and 1380 cm^{-1}. The weak peak at 1540 cm^{-1} is ascribable to the C=N stretching and peaks at 1460 and 1380 cm^{-1} to N=N stretching as reported by earlier workers[45,46]. On the basis of elemental analysis and i.r. spectrum, two isomeric structures can be written for the compound, m.p. 169°. It has either 6-aza-B-homo-5∝-cholestano[6,7-d] tetrazole structure (CCCVI) or the alternative 7-aza-B-homo-5∝-cholestano[6,7-d]tetrazole structure (CCCVII). A clear distinction between the two was made possible with the help of n.m.r. spectrum of this compound.



(XXXIII)



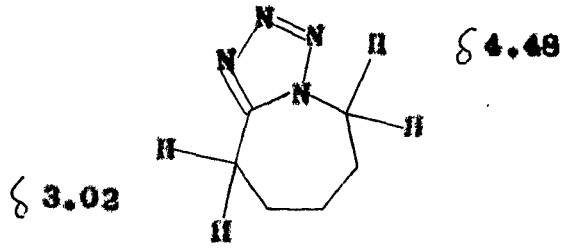
(CCCVI)



(CCCVII)

It has been reported by DiMaio and Permutti[81] that n.m.r. spectrum of the tetrazole (CCCVIII) exhibits a two proton multiplet at δ 4.48 which is ascribable to the methylene group directly

attached to the ring nitrogen atom and another two proton multiplet at δ 3.02 due to the methylene group adjacent to C=N fragment of the tetrazole moiety.



(CCCVIII)

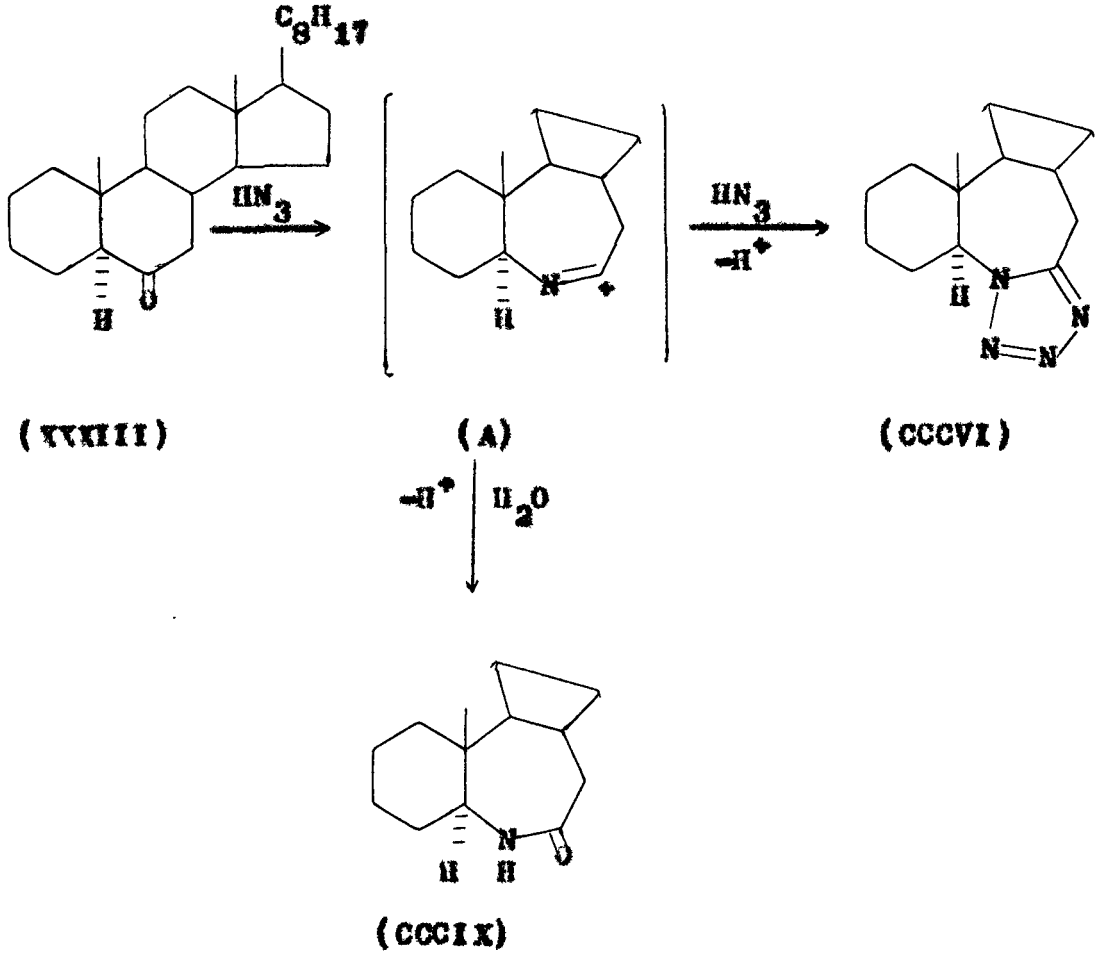
The n.m.r. spectrum of the compound, m.p. 169°, exhibited a double doublet for 1 proton at δ 4.26 which is easily ascribable to C5-$\underline{\alpha}$H (axial) in the 6-aza structure (CCCVI). In case of the alternative structure (CCCVII), C7a protons (C7a-$\underline{H}_2$-N-) would have appeared around δ 4.2-4.5. The appearance of C5-axial proton signal as a double doublet can be reasonably explained by considering that C5-axial proton interacts with C4-axial (β) proton to give a doublet with J value of 10 Hz (A/B ring junction trans) and with C4-equatorial (α) proton to give another doublet with J value of 7 Hz and thus a four line pattern is obtained. The striking features, however, of this spectrum were (i) the non-appearance of C7a protons together and (ii) the diamagnetic shift of the C13 methyl resonance. The C7a protons were expected to appear together at about δ 3.0 as shown for (CCCVIII)[81]. However,

an unequal doublet for only 1 proton was observed at δ 3.21. The coupling constant (15 Hz) between the two halves of the doublet suggested the dominance of geminal over vicinal coupling. Possibly, the C8,7 tetrazole ring affects the geometry of ring B to such an extent that the dihedral angle between C8-βH and one of the C7a protons approaches $\sim 90^{0}$ which may satisfactorily account for the dominance of geminal coupling. It is reasonable to believe that geometrical disposition of ring B might have allowed the other C7a proton to remain uninfluenced by the magnetic effect of tetrazole ring whose signal merges with the methylene envelope. This contention was also strengthened by the fact that the upfield part of the doublet was taller indicating thereby that it is being coupled with a proton resonating at a higher field. A cursory examination of the Dreiding model of tetrazole (CCCVI) revealed that, in fact, the dihedral angle between the planes of C8-βH and one of the C7a protons (equatorial like) is near about 90^{0} due to which no vicinal coupling is observed. This proton is now geminally coupled with the other C7a proton (axial like) to give a coupling constant of 15 Hz.

Another interesting feature of this spectrum is the unusual diamagnetic shift of the signal for C13-methyl protons (δ 0.43). A double bond at C7 in the steroid framework is reported[80] to cause shielding of C13-methyl protons. Similarly, C8,7 tetrazole with its sp^2 hybridized C7 may, by way of its geometry altering

effect, force the C13-methyl to 'see' more of the molecule, hence the notable diamagnetic shift. Other methyl signals were observed at δ 0.90, 0.81 and 0.63 (remaining methyl groups).

The compound, m.p. 175° was identified as 6-aza-B-homo-5α-cholestan-7-one (CCCIX) by direct comparison with an authentic sample[82]. The formation of the lactam (CCCIX) further supports the 6-aza structure (CCCVI) for the tetrazole, m.p. 169°, because of the common intermediate (A) involved in such reactions.

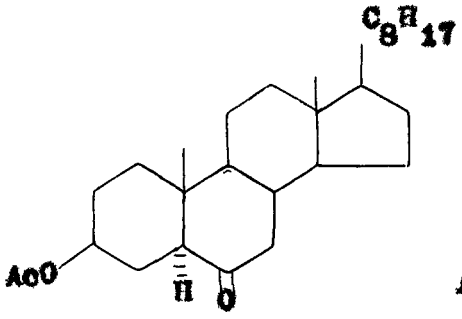


(XXXIII) (A) (CCCVI)

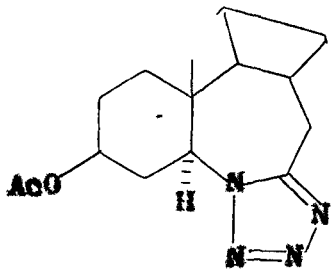
(CCCIX)

Reaction of 3β-acetoxy-5α-cholestan-6-one (XXXIV) with an excess of hydrazoic acid

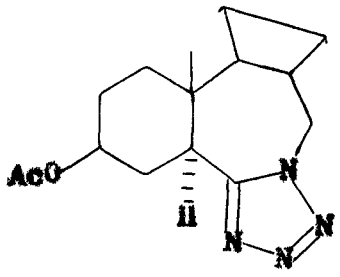
3β-Acetoxy-5α-cholestan-6-one (XXXIV) on similar treatment with an excess of hydrazoic acid afforded two compounds, m.pts. 185° and 170°.



(XXXIV)



(CCCX)



(CCCXI)

Characterization of the compound, m.p. 185° as 3β-acetoxy-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCX)

The compound, m.p. 185° analysed for $C_{29}H_{48}N_4O_2$ which is a clear evidence of the presence of tetrazole moiety in the compound. The i.r. spectrum of compound, m.p. 185° showed bands at 1720s (CH_3-COO), 1525 (C=N), 1455, 1360 (N=N) and 1240 cm^{-1} (acetate). Its n.m.r. spectrum gave a broad peak at δ 4.78 ($w\frac{1}{2}$ 14 Hz) integrating for 1 proton which is ascribable to C3-αH (axial). The 6-aza structure (CCCX), in preference to the alternate 7-aza structure (CCCXI), was supported on the basis of a charac-

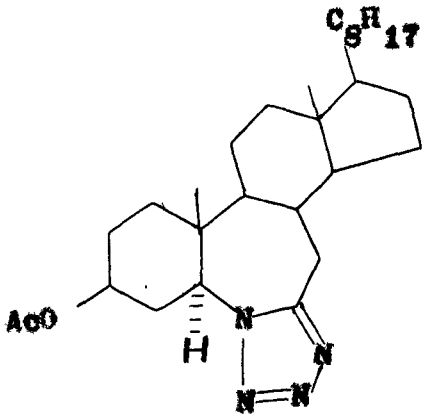
teristic double doublet at δ 4.45 ($J_{a,a}$ 14 Hz; $J_{a,e}$ 7 Hz) for 1 proton which can be ascribed to C5-αH (axial). One of the C7a protons again appeared as a doublet of unequal heights at δ 3.4 (J 15 Hz). It bears striking similarity to the doublet observed in the n.m.r. spectrum of the tetrazole (CCCVI). A sharp singlet for 3 protons at δ 2.06 was assigned to methyl group of acetate function (CH_3-COO-). The diamagnetic influence of the tetrazole ring on the C13-methyl signal was again in evidence as this signal appeared appreciably at higher field (δ 0.55) but lower with respect to C13 methyl signal in (CCCVI). The presence of C3-acetate group might have something to do with the downfield shift of the key peaks when compared to (CCCVI). Other signals were observed at δ0.91, 0.83 and 0.65 (remaining methyl groups).

The compound, m.p. 170° which analysed for five nitrogen atoms remained uncharacterized due to its small quantity.

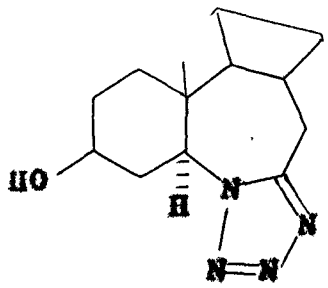
3β-Hydroxy-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCXII)

The acetate group of the tetrazole (CCCX) was hydrolysed to give the hydroxy tetrazole (CCCXII), m.p. 190°, which analysed for $C_{27}H_{46}N_4O$. The i.r. spectrum of (CCCXII) showed bands at 3400br (OH), 1540, 1480 and 1390 cm^{-1}(C=N, N=N). Its n.m.r. spectrum gave a characteristic double doublet at δ 4.33 ($J_{a,a}$ 12 Hz; $J_{a,e}$ 7 Hz) for 1 proton which was assigned to C5-αH (axial). A broad signal at δ 3.75 ($W_{\frac{1}{2}}$ 12 Hz) integrating for 1 proton was

ascribable to C3-αH (axial). As previously noted, an unequal doublet (J 15 Hz) appeared at δ 3.38 for one of the C7a protons while the other proton merged with the methylene envelope. The hydroxy proton appeared at δ 2.2 as indicated by D_2O addition. Here again C13 methyl signal was observed at appreciably high field (δ 0.52). Other signals were seen at δ 0.91, 0.81 and 0.63 (remaining methyl groups).



(CCCX)

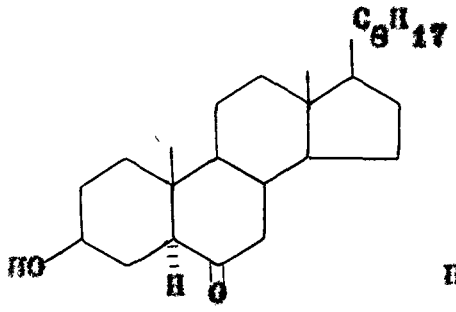


(CCCXII)

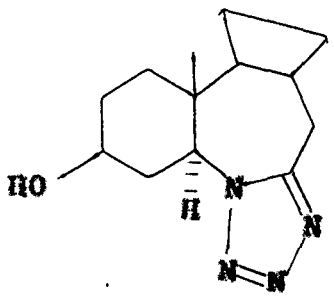
<u>Reaction of 3β-hydroxy-5α-cholestan-6-one (CCLIII) with an excess of hydrazoic acid</u>

3β-Hydroxy-5α-cholestan-6-one (CCLIII) was treated with an excess of hydrazoic acid. Usual work up and subsequent column chromatography over silica gel afforded two compounds m.pts. 190° and 277-78°. The compound, m.p. 190° was found to be the hydroxy tetrazole (CCCXII) because their m.m.p. showed

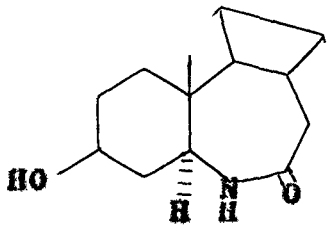
no depression and further its i.r. spectrum and t.l.c. were found identical with the hydroxy tetrazole (CCCXII) obtained from acetate hydrolysis of (CCCX). The compound, m.p. 277-78° was identified as 3β-hydroxy-6-aza-B-homo-5α-cholestan-7-one (CCCXIII) by direct comparison with an authentic sample[63].



(CCLIII)



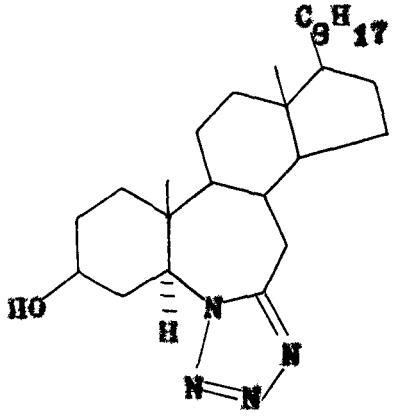
(CCCXII)



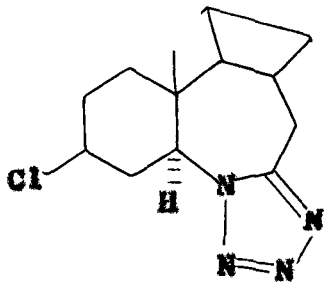
(CCCXIII)

The hydroxy tetrazole (CCCXII) on treatment with thionyl chloride afforded the chlorotetrazole (CCCXIV), m.p. 195° (Positive Beilstein test)of [84], and analysed for $C_{27}H_{45}N_4Cl$. The i.r. spectrum showed the characteristic bands for tetrazole moiety at 1540, 1470, 1380 (C=N, N=N) and 730 cm^{-1} (C-Cl). In the n.m.r. spectrum the characteristic double doublet for C5 proton (axial) merged with the C3 proton at δ 4.33 which appeared as a distorted doublet of doublet (C5-$\underline{H}$, $J_{a,a}$ 10 Hz; $J_{a,e}$ 6 Hz and C3-$\underline{H}$; $W_{\frac{1}{2}}$ 12 Hz). One of the C7a protons again appeared as a doublet of unequal height at δ 3.4 (15 Hz) while the other proton

got merged with the methylene envelope. A marked diamagnetic influence of the tetrazole ring on the C13 methyl signal was again observed, as the signal appeared at a high field (δ 0.53). Other signals were seen at δ 0.93, 0.83 and 0.66 (remaining methyl groups).



(CCCXII)

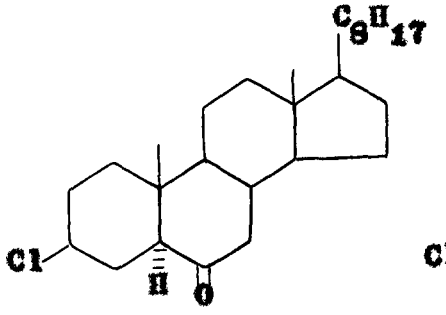


(CCCXIV)

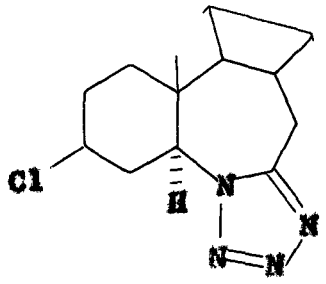
Reaction of 3β-chloro-5α-cholestan-6-one (CCXLIX) with an excess of hydrazoic acid

3β-Chloro-5α-cholestan-6-one (CCXLIX) on similar treatment with an excess of hydrazoic acid afforded, two compounds, m.pts. 195° and 150°. The compound m.p. 195° (Positive Beilstein test) was found identical with the chloro tetrazole (CCCXIV) on the basis of its m.p. and m.m.p. (no depression observed) and comparison of its i.r. spectrum and t.l.c. with chlorotetrazole (CCCXIV) obtained previously. The compound, m.p. 150° was identified as 3β-chloro-6-aza-B-homo-5α-cholestan-7-one (CCCXV) by direct

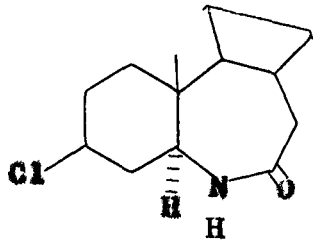
comparison with an authentic sample[85].



(CCXLIX)

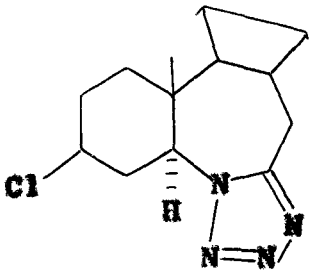


(CCCXIV)

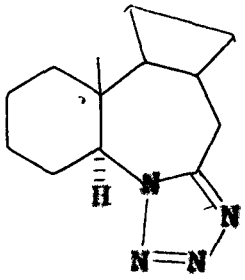


(CCCXV)

The chlorotetrazole (CCCXIV) on sodium-pentyl alcohol reduction furnished the 6-aza tetrazole (CCCVI) and this further supports the structure (CCCXIV) for compound, m.p. 195°.



(CCCXIV)

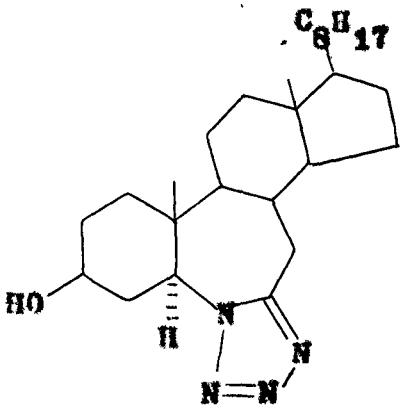


(CCCVI)

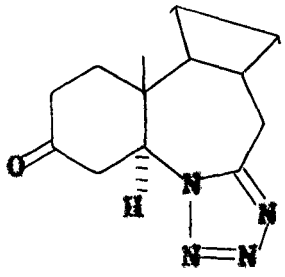
3-Oxo-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCXVI)

The hydroxy tetrazole (CCCXII) on Jones' oxidation[71] afforded the 3-oxotetrazole (CCCXVI), m.p. 205°, which analysed for $C_{27}H_{44}N_4O$. Its i.r. spectrum showed bands at 1722s (C=O),

1530, 1460 and 1360 (C=N, N=N). In its n.m.r. spectrum, the C5 proton appeared as a fine doublet of doublet centred at δ 4.66 (C5-$\underline{H}$, $J_{a,a}$ 13 Hz; $J_{a,e}$ 6 Hz). The downfield shift of C5-$\underline{H}$ in comparison to tetrazole (CCCXII) may be due to the magnetic influence of C3 oxo group which also seems to influence the resonance of C7a methylene protons, both of which appear as a multiplet centred at δ 3.50 in contrast to other tetrazoles (CCCVI), (CCCX), (CCCXII) and (CCCXIV) where only 1 proton appeared in that region. The C3-oxo group also seems to counter the diamagnetic influence of the tetrazole ring on the resonance of C13 methyl protons, which is brought down to its normal position (δ 0.68). Other signals were observed at δ 0.91, 0.81 and 0.73 (remaining methyl groups).



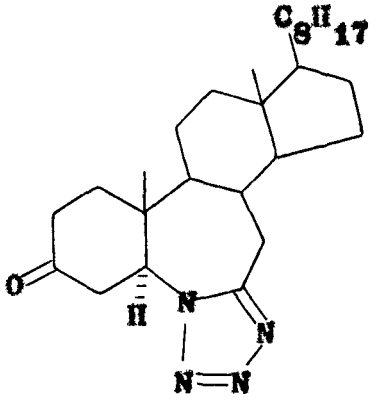
(CCCXII)



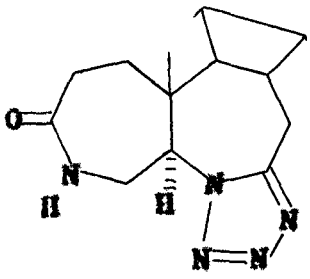
(CCCXVI)

4,6-Diaza-A,B-bishomo-3-oxo-5∝-cholestano[6,7-d]tetrazole (CCCXVII)

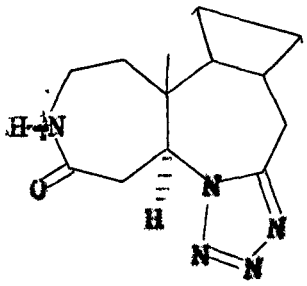
The 3-oxotetrazole (CCCXVI) on treatment with a unimolecular quantity of sodium azide in benzene-sulphuric acid led to 4,6-diaza-A,B-bishomo-3-oxo-5∝-cholestano[6,7-d]tetrazole (CCCXVII) m.p. 260°. The compound, m.p. 260° analysed for $C_{27}H_{45}N_5O$ and its i.r. spectrum exhibited peaks at 3340, 3200 (NH), 1690, 1640 (CONH), 1540, 1470 and 1380 cm^{-1} (C=N, N=N). A clear distinction between (CCCXVII) and its isomer (CCCXVIII) was made possible with the help of n.m.r. spectrum of this compound.



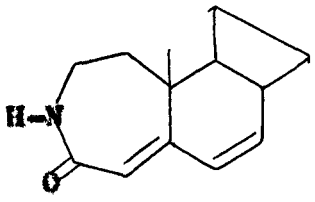
(CCCXVI)



(CCCXVII)



(CCCXVIII)



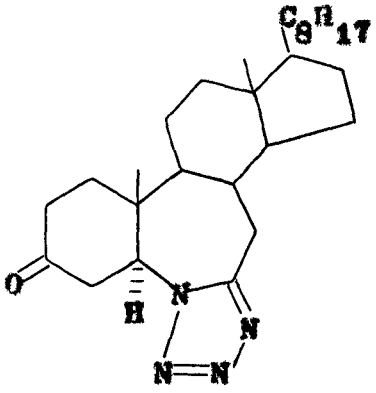
(CCCXIX)

The n.m.r. spectrum gave a signal at δ 7.0 integrating for 1 proton and this peak disappeared on D_2O addition. Therefore, it is easily ascribable to the proton bonded to nitrogen (CONH). A broad peak centred at δ 4.66 for 1 proton was assigned to C5 proton. The alternative structure (CCCXVIII) is excluded on the basis of a multiplet at δ 4.06 for 2 protons which is assignable to C4a methylene protons. This signal was simplified on deuterium exchange and from the multiplicity of the modified signal, it was evident that NH group had in its vicinity CH_2-CH grouping. In the alternate structure (CCCXVIII), deuterium exchange is likely to produce relatively 'broader' peaks compared to (CCCXVII) because in the former, C2 methylene will be split by C1 methylene. For further support, the n.m.r. spectrum of (CCCXVII) was compared with that of 3-aza-A-homocholesta-4a, 6-dien-4-one (CCCXIX)[96] where dissimilarity in the multiplicity of $NHCH_2$ signal before and after deuterium exchange was noted. Like the oxotetrazole (CCCXVI), both the C7a protons again appeared together as a multiplet at δ 3.41 (C7a-H_2). The C13 methyl signal in this compound again appeared at a higher field (δ 0.45). Other signals were seen at δ 0.91, 0.81 and 0.65 (remaining methyl groups).

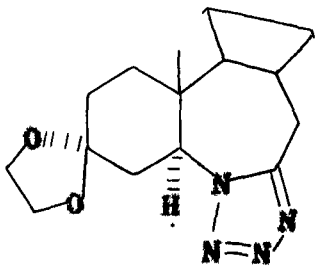
3,3-Ethylenedioxy-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCXX)

Reaction of 3-oxotetrazole (CCCXVI) with ethylene glycol

in the presence of p-toluenesulphonic acid monohydrate as catalyst furnished 3,3-ethylene dioxy-6-aza-B-homo-5∝-cholestano[6,7-d] tetrazole (CCCXX), m.p. 195°. The compound, m.p. 195° analysed for $C_{29}H_{50}N_4O_2$ and its i.r. spectrum gave bands at 1535, 1455, 1390 (C=N, N=N), 1180, 1100 and 1030 cm^{-1} (C-O). The n.m.r. spectrum of the compound (CCCXX) exhibited a doublet of doublet for 1 proton at δ 4.46 ($J_{a,a}$ 12 Hz; $J_{a,e}$ 6 Hz) which is easily ascribable to C5-∝H (axial) (ring junction A/B trans). A sharp signal integrating for 4 protons at δ 3.96 could be ascribed to the protons of the dioxolane ring (O-CH_2-CH_2-O). In this case too, an unequal doublet for one of the C7a protons was observed at δ3.42. The coupling constant (15 Hz) between the two halves of doublet clearly supports the dominance of geminal over vicinal coupling. The C13 methyl group was again found at a higher field (δ 0.51). Other signals were seen at δ 0.9, 0.81 and 0.63 (remaining methyl groups).



(CCCXVI)



(CCCXX)

The important n.m.r. signals for the tetrazoles are tabulated below (Table IV).

Table - IV

(N.M.R. Values in δ)

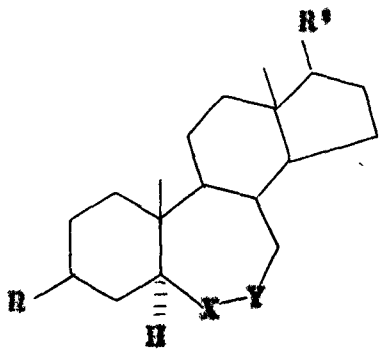
Compound	C5-αH	C7a	C13-CH_3
(CCCVI)	4.26	3.21(1H)	0.43
(CCCX)	4.45	3.40(1H)	0.55
(CCCXII)	4.33	3.38(1H)	0.52
(CCCXIV)	4.66	3.40(1H)	0.53
(CCCXVI)	4.66	3.50(2H)	0.68
(CCCXVII)	4.66	3.41(2H)	0.45
(CCCXX)	4.46	3.42(1H)	0.51

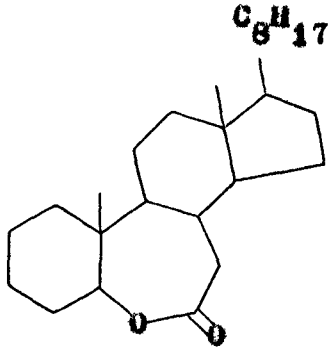
It is pertinent to summarise the marked features of our study. The nitrogen insertion invariably occurred between C5, C6 via an imidocarbonium ion intermediate (A) which gives tetrazoles and lactams by competing reaction with hydrazoic acid and water, respectively. The most conspicuous features of tetrazoles were exhibited in their n.m.r. spectra. The appearance of a 1 proton double doublet between δ 4 to 5 is characteristic of the C5-axial (α) proton with J 14 and 7 Hz. The signal is influenced by C3-substituents. A 3-oxo group in (CCCXVI) and

(CCCXVII) causes it to move more downfield than other C3-substituents. Only one of the C7a protons appeared downfield as a geminally coupled doublet (J ca 15 Hz) while the other proton coalescing with the methylene envelope. This signal is also affected by C3-substitution. A comparison of the n.m.r. data of tetrazoles (CCCVI), (CCCX), (CCCXII), (CCCXIV) and (CCCXX) discloses this fact. In (CCCXVI) and (CCCXVII), the C3-oxo group deshields the other C7a proton also and both appear together. Finally the most distinctive feature is the upfield shift of C13 methyl resonance caused by the tetrazole ring. This property fails to manifest itself in the spectrum of (CCCXVI) where it is offset by the paramagnetic effect of the C3-oxo group. Interestingly it may be noted that the tetrazole ring does not seem to influence the C10 methyl resonance as observed[51] for 3-aza-A-homo-4a-eno[3,4-d] and 7a-aza-B-homo-5/4-eno[7a,7-d] tetrazoles where it is shifted downfield. This might well be due to more subtle interplay of molecular distortion caused by the fusion of tetrazole ring.

Mass Spectrometry of Steroidal 7-Oxalactones

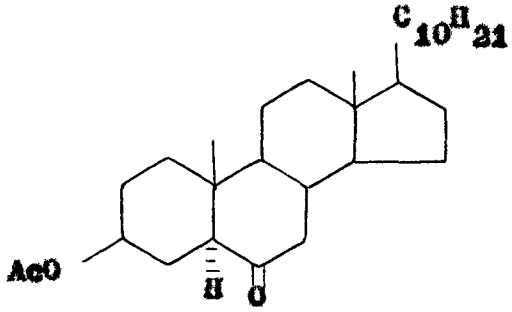
One of the previous communications from these laboratories was concerned with mass spectral study of several "6-oxalactones" (XXXV, CCXLIII-CCXLV).[68]





(XXXV) R,H; R',C_8H_{17}; X,O; Y,CO
(CCXLIII) R,Cl;R',C_8H_{17}; X,O; Y,CO
(CCXLIV) R,Br;R',C_8H_{17}; X,O; Y,CO
(CCXLVIII) R,H; R',C_8H_{17}; X,CO; Y,O
(CCLV) R,OAc;R',$C_{10}H_{21}$; X,CO; Y,O
(CCLVI) R,OAc;R',$C_{10}H_{21}$;X,O; Y,CO
(CCLXVIII) R,Cl;R',C_8H_{17};X,CO; Y,O.
(CCLXIX) R,Br;R',C_8H_{17};X,CO; Y,O

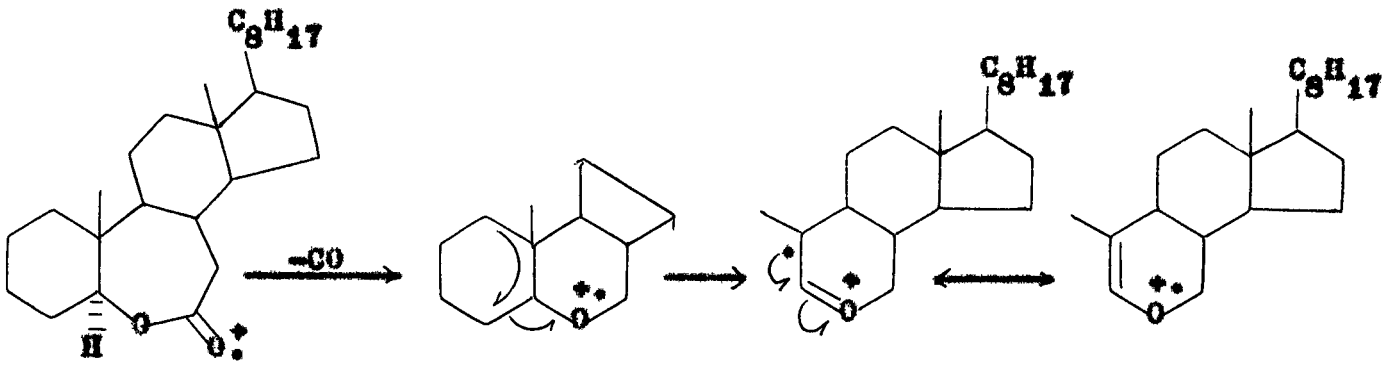
(CCXLV)



(CCLII)

These spectra were conspicuous by an intense peak at m/e 318 which was considered of "partial" diagnostic value in the characterisation of such lactones. A mechanism was proposed for the genesis of this fragment ion as shown in Scheme-24.

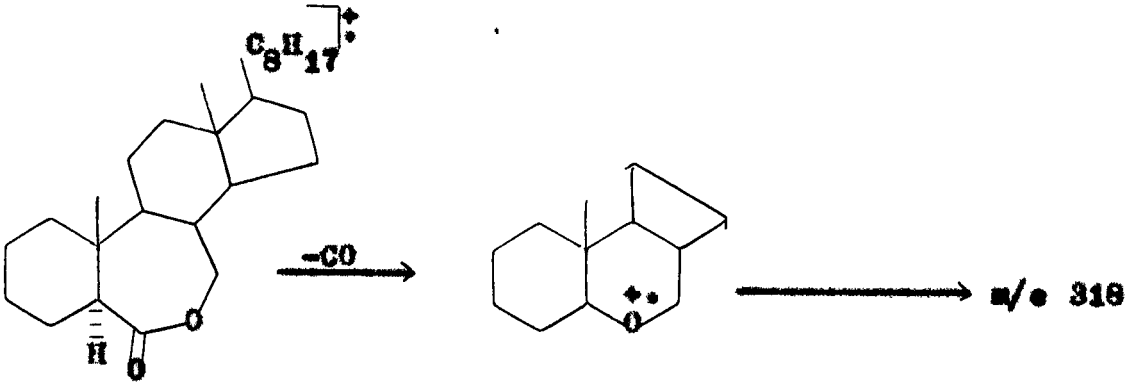
Scheme - 24



(XXXV') (a) (b) m/e 318 ($C_{22}H_{38}O$) (c)

It was pointed out that since ion a can also be obtained (atleast theoretically) from the isomeric 7-oxalactone, such as (CCXLVIII), the peak at m/e 318 can be only of limited diagnostic value in the characterisation of 6-oxalactones (XXXV, CCXLIII-CCXLV)(Scheme-25).

Scheme - 25



(CCXLVIII) (a) m/e 318

In view of the above remarks it was considered desirable to substantiate the mechanism proposed in Scheme-24 by studying the mass spectrum of a steroidal ring B ϵ -lactone having a different side chain. The difference in the side chain will be reflected in the fragment ion analogous to the ion m/e 318 of the cholestane series. For this purpose, it was planned to obtain 6-oxa-B-homo-7-oxo-5α-β-sitostanyl acetate (CCLVI) by the usual Baeyer-Villiger oxidation of the corresponding 6-ketone (CCLII). It was also 'wishfully' thought to obtain 7-oxa-B-homo-5α-cholestan-6-one (CCXLVIII), and its 3β-chloro (CCLXVIII) and 3β-bromo (CCLXIX) analogues and study their mass spectra so as to obviate or substantiate doubt expressed in Scheme 25.

In an attempt to obtain (CCLVI), the ketone, 6-oxa-5α-β-sitostanyl acetate (CCLII) was subjected to usual Baeyer-Villiger oxidation and this resulted in the formation of (CCLVI) along with its isomer, 7-oxa-B-homo-6-oxo-5α-β-sitostanyl acetate (CCLV). Similarly it was possible for us to obtain both the isomeric lactones (XXXV and CCXLVIII), (CCXLIII and CCLXVIII) and (CCXLIV and CCLXIX) from respective 6-ketones. Isolation and characterisation of these isomeric lactones have already been described in an earlier section of the thesis.

The present chapter is concerned with the examination of the mass spectra of the isomeric ϵ -lactones (XXXV, CCXLVIII), (CCXLIII, CCLXVIII) and (CCLV, CCLVI). It must be admitted that

the previous study on "6-oxalactones" (XXXV, CCXLIII, CCXLIV) was made on samples of doubtful purity. Therefore, it was considered desirable to reexamine the spectra obtained with reliable samples of (XXXV, CCXLIII, CCXLIV).

The mass spectra of the isomeric lactones, 7-oxa-5α-B-homocholestan-6-one (XXXV) and 6-oxa-5α-B-homocholestan-7-one (CCXLVIII) (Figs. 1 and 2, respectively) were examined together and salient points of similarity and difference noted. (It must be pointed out that the mass spectra of (XXXV) obtained in this and previous studies were fairly similar and masking of (XXXV) by (CCXLVIII) in the previous study was not too obvious).

The mass spectrum of (CCXLVIII) gave molecular ion peak at m/e 402 ($C_{27}H_{46}O_2$) followed by significant peaks at m/e 387, 384, 372 (M-30), 334, 318, 317, 304, 289, 263, 262, 261, 260, 247, 232, 218, 177, 149, 141, 135, 126, 123, 122, 121 and lower mass peaks.

For the sake of comparison the mass spectral values of (XXXV) are also recorded: M 402, m/e 387, 384, 374, 359, 356, 345, 342, 341, 333, 332, 331, 318 (base peak), 317, 306, 303, 289, 262, 247, 245, 233, 229, 219, 216, 205, 203, 201, 178, 167, 163, 152, 149, 135, 121 and lower mass peaks.

Even a cursory glance at the mass spectra of (XXXV) and (CCXLVIII) brings out the salient points of difference between the two, to the extent that mass spectrometry may be claimed to

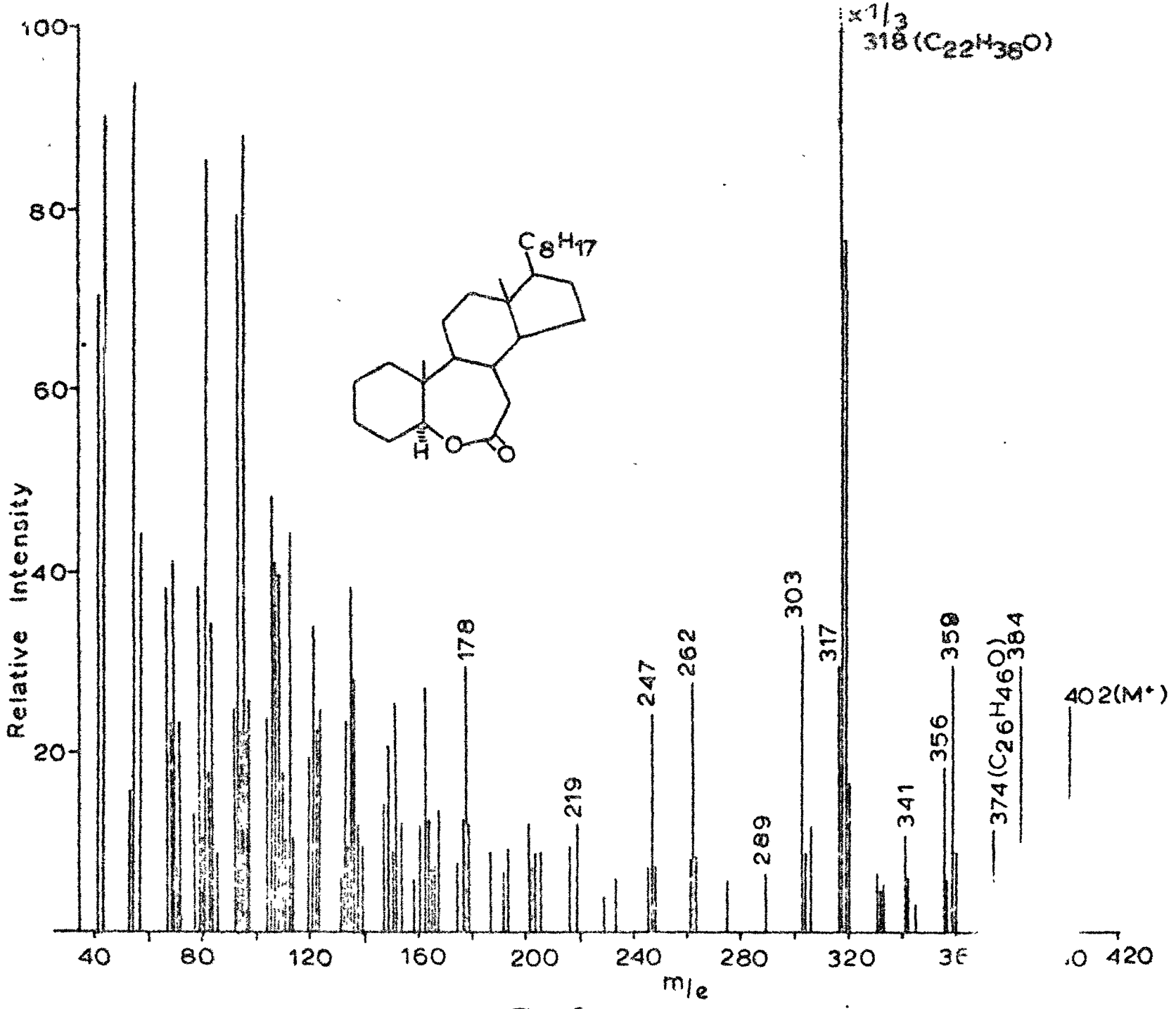

100
80
60
40
20
Relative Intensity
40
80
120
160
200
240
280
320
m/e
×1/3
318 (C22H38O)
C8H17
178
219
247
262
289
303
317
341
356
358
374 (C26H46O)
384
402 (M+)
420

Fig.-2

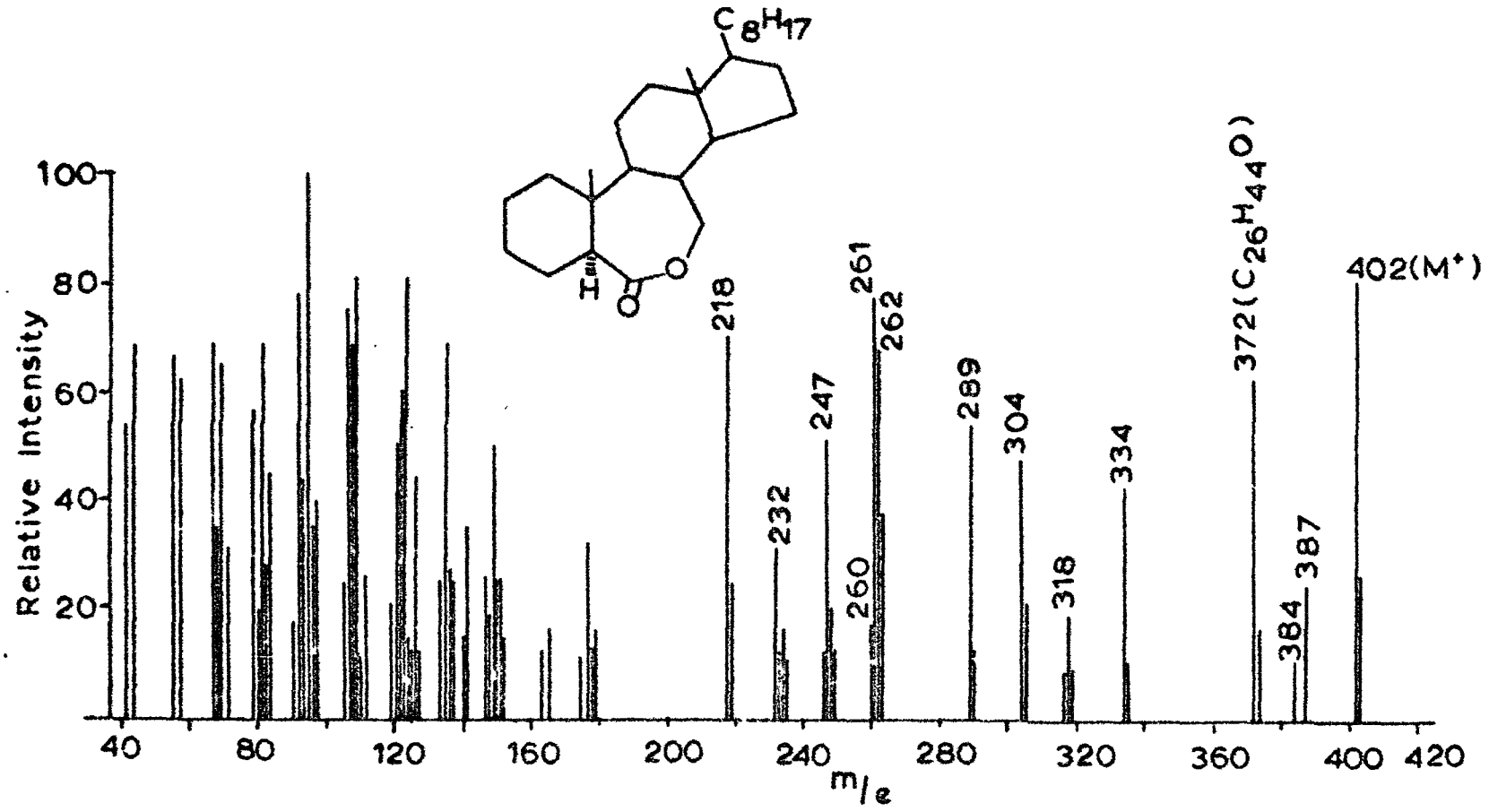

C8H17
H
O
O
Relative Intensity
100
80
60
40
20
40
80
120
160
200
240
280
320
360
400
420
m/e
218
232
247
260
261
262
289
304
318
334
372(C26H44O)
384
387
402(M+)

Fig.-1

offer a simple method for differentiating between the isomeric lactones such as (XXXV) and (CCXLVIII). The most notable points of difference between the mass spectra of (XXXV) and (CCXLVIII) can be summarized as in Table-V. These differences are of diagnostic value since the other isomeric lactones (CCXLIII)and CCLXVIII) and (CCLV and CCLVI) also exhibited analogous behaviour.

Table - V

(XXXV)	(CCXLVIII)
a. M-CO	M-CH_2O; no M-CO
b. m/e 318 base peak	m/e 318; almost negligible
c. m/e 262 prominent	m/e 262, 261, almost of the same strength m/e 260
d. -	M-68 (loss of C_3,C_4,C_5,C_6)

The formation of some of the important fragment ions, which have a bearing on the structure elucidation and differentiation, has been rationalised in the schemes given below. Since the mass spectrum of (XXXV) has been studied earlier to a considerable degree of satisfaction, no attempt is being made to reinterpret the spectrum of (XXXV). It should be pointed out that the proposed mechanisms are tentative in the absence of mass spectra of deuterated analogues of (CCXLVIII).

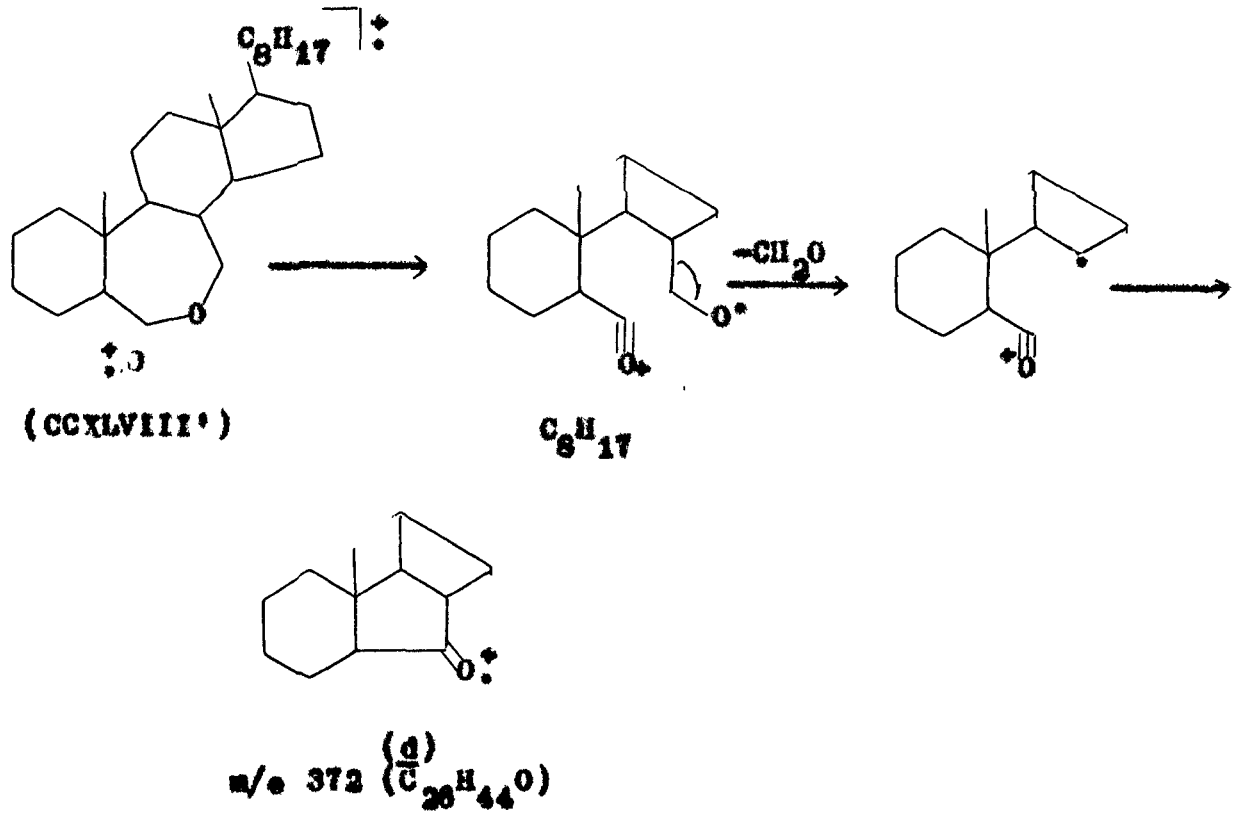
m/e 387 and 384

These peaks represent the loss of a methyl group and water, respectively from the molecular ion of (CCXLVIII).

m/e 372 ($M-CH_2O$)

This significant ion can be shown to arise by the loss of a molecule of formaldehyde or by two consecutive methyl losses from the molecular ion. That the former is the case has been substantiated by its composition ($C_{26}H_{44}O$) as determined by accurate mass measurement. The loss of formaldehyde from the molecular ion can be shown according to Scheme-26.

Scheme - 26



The loss of formaldehyde from suitably constructed lactones, under electron impact, is a well known phenomenon[87]. It should, however, be pointed out that there was no evidence for the loss of CO from the molecular ion (CCXLVIII') as it occurred in the case of (XXXV). The loss of formaldehyde from (CCXLVIII) is extremely useful for characterisation purpose. The loss of formaldehyde from (XXXV) is not observed since there is no rationale for such a loss form (XXXV). However, one would have anticipated loss of a ketene molecule from (XXXV) (Scheme-27) to give a fragment ion m/e 360 but there seems to be no convincing evidence for this.

Scheme - 27

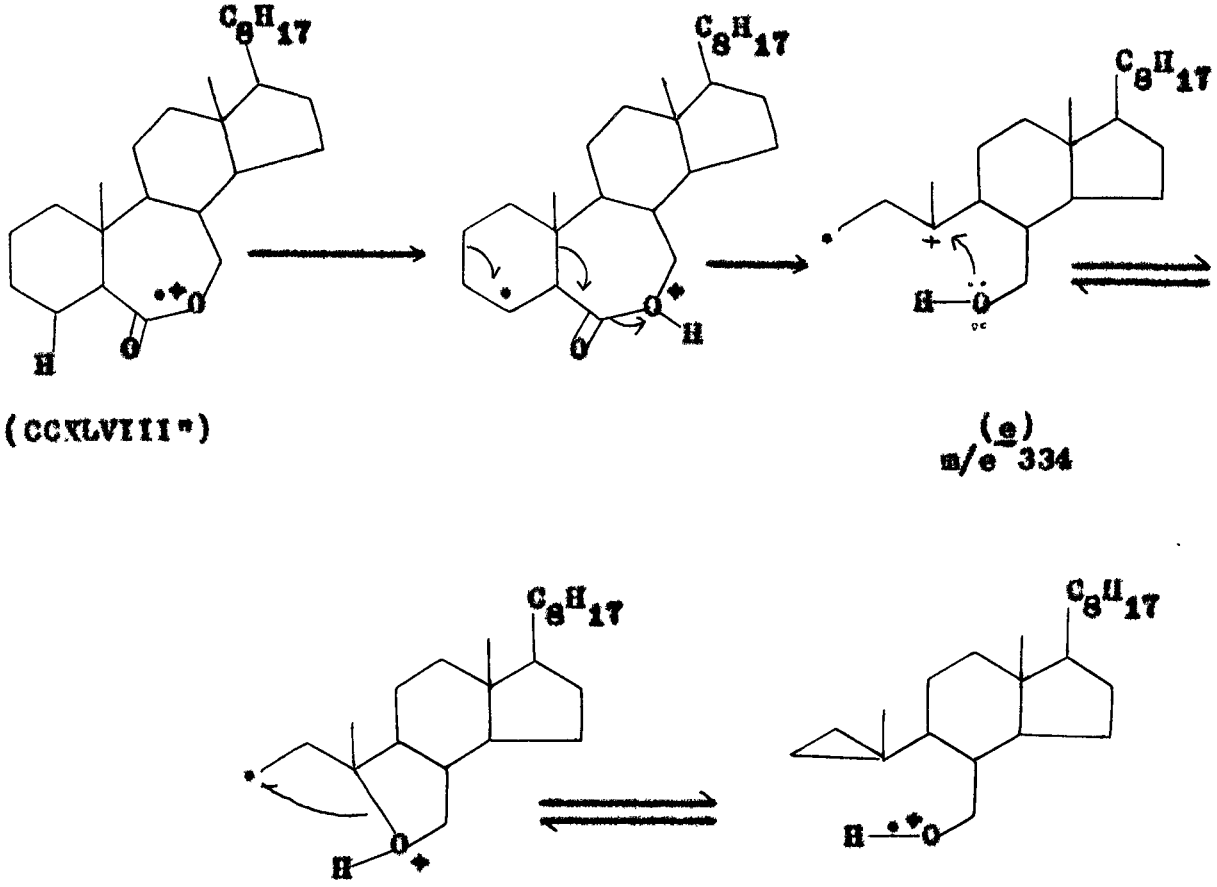
(XXXV') m/e 360

m/e 334

This fragment ion (M-68) obviously results by the loss of C_3-C_6 from the molecular ion. This loss also occurred in (CCLXVIII) and (CCLVI) giving fragment ions m/e 334 and m/e 362, respectively. This mode of fragmentation thus seems to be peculiar

of 7-oxa lactones and therefore may be considered of diagnostic importance. The mechanism of this cleavage is shown in Scheme-23.

Scheme - 28



m/e 318

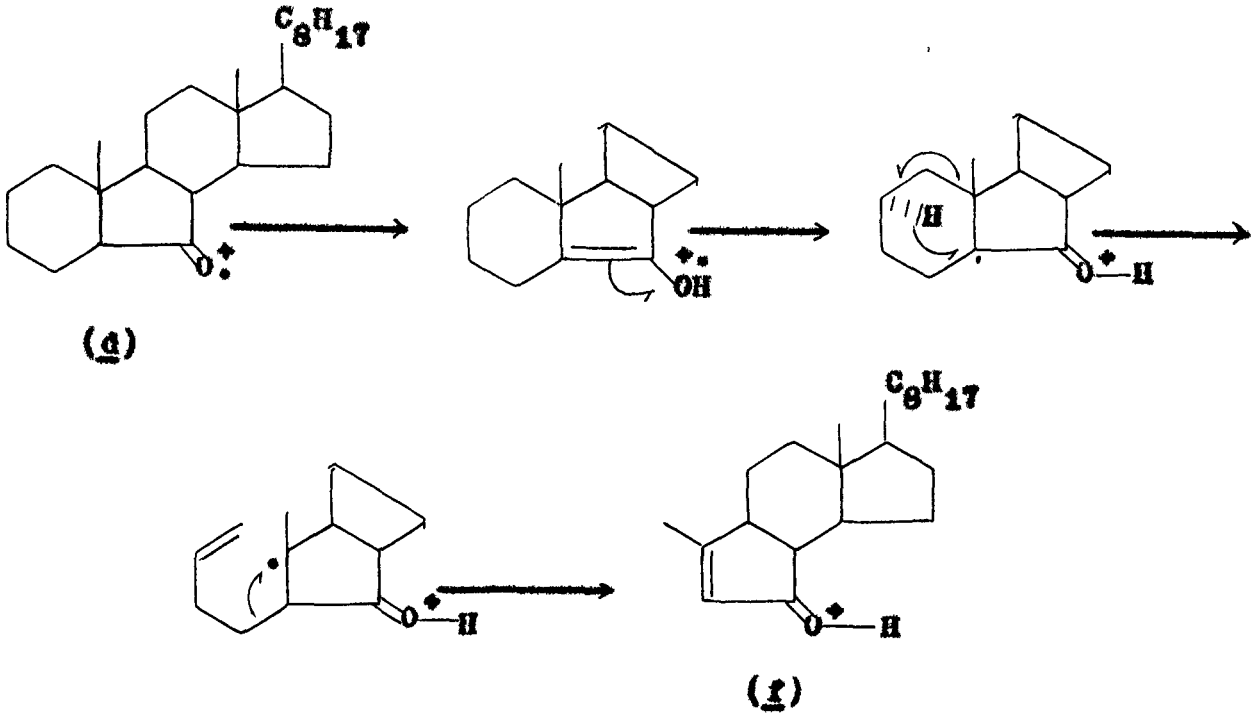
The fragment ion peak at m/e 318 is weak (in the spectrum of (XXXV) the base peak is at m/e 318). It is possible to suggest that this fragment ion may be derived from (CCXLVIII) as outlined in Scheme-25. However, this does not seem to be the case as in

other lactones (CCLXVIII) and (CCLV) there is no analogous peak. In view of the comparative data available for (CCLXVIII) and (CCLV) it is suggested that the peak at m/e 318 could be due to trace impurity of (XXXV) in the sample of (CCXLVIII).

m/e 317

This peak could be due to either m/e 318-H, M-85 or derived from the ketonic species d. As the relative abundance of the fragment ion m/e 317 with respect to m/e 318 is higher than in (XXXV) it is suggested that it does not come alone from m/e 318-H process; it could therefore be considered as originating from the molecular ion by the loss of mass 85 or more attractively from the ketonic species d by a process analogous to that described for steroidal 6-ketones[88] giving fragment ion m/e 331 (Scheme-29).

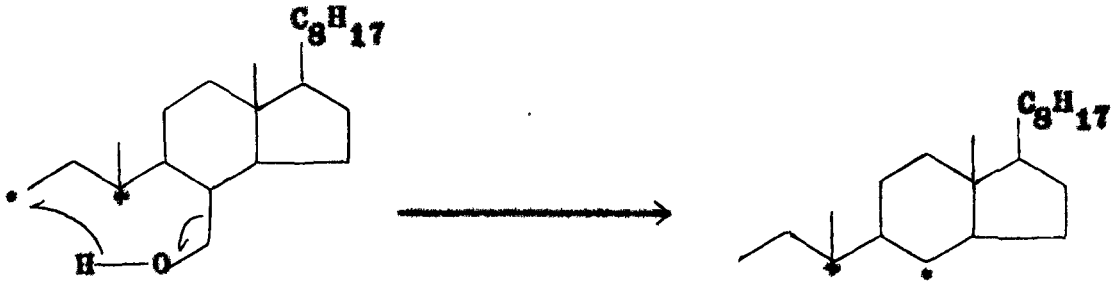
Scheme - 29



m/e 304

Apparently the fragment ion m/e 304 results by the loss of a molecule of formaldehyde from the ion m/e 334 and its formation can be shown according to Scheme-30.

Scheme - 30



m/e 334 m/e 304

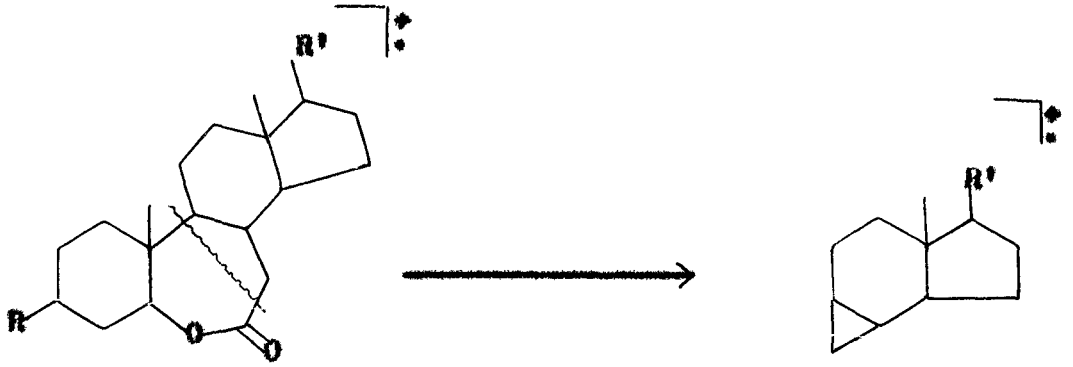
m/e 289

This fragment ion may either be derived from the ion m/e 304 by the loss of a methyl group or from the molecular ion by the loss of side chain (C_8H_{17}; mass unit 113) or by both the processes.

m/e 262

One of the striking features of the mass spectra of 7-oxalactones (CCXLIII) and (CCLXVIII) is a bunch of peaks at m/e 262, 261 and 260 (in the order 261 262 260) and the analogous peaks at m/e 290, 289, and 288 (in the same abundance

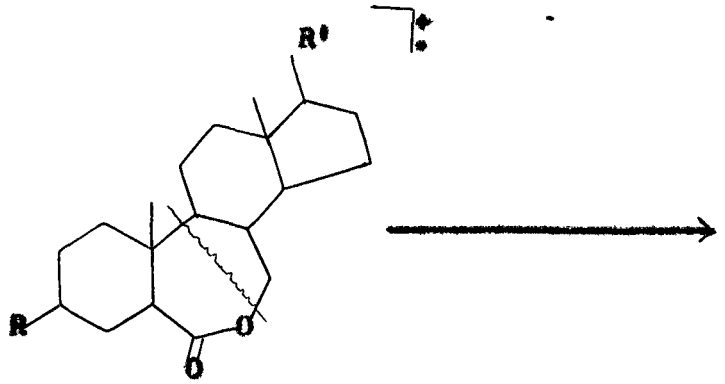
order) in the spectrum of (CCLV). From these comparative data, it becomes obvious that these fragment ions are composed of C7a, rings C,D and the side chain. The spectra of (XXXV) and (CCXLIII) showed strong peak at m/e 262 whereas the spectrum of (CCLVI) gave the corresponding peak at m/e 290. This again shows that the fragment ion m/e 262 from (XXXV) and (CCXLIII) and the ion m/e 290 from (CCLVI) consist of C7a, rings C,D and the side chain.



(h)

(XXXV') R,H; R',C_8H_{17}
(CCXLIII') R,Cl; R',C_8H_{17}
(CCLVI') R,OAc; R',$C_{10}H_{21}$

m/e 262 R', C_8H_{17}
m/e 290 R', $C_{10}H_{21}$

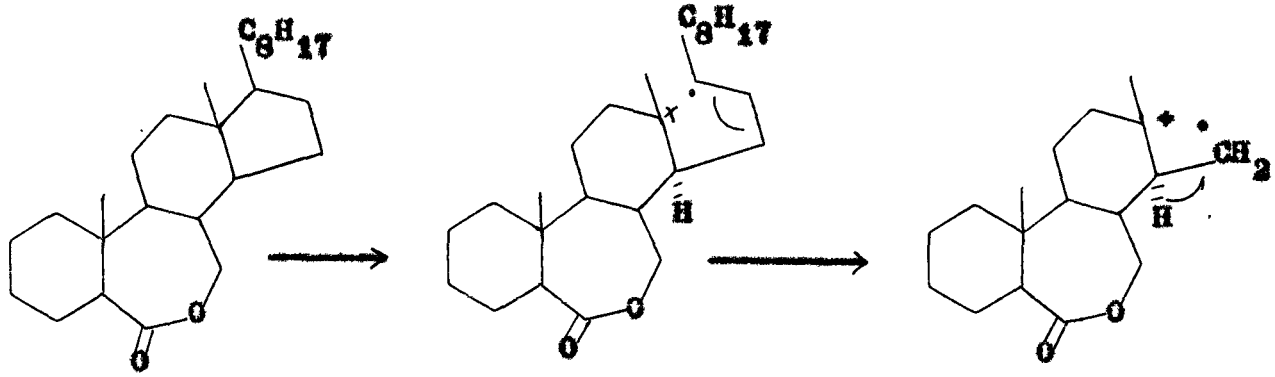


m/e 262 R', C_8H_{17}
m/e 290 R', $C_{10}H_{21}$

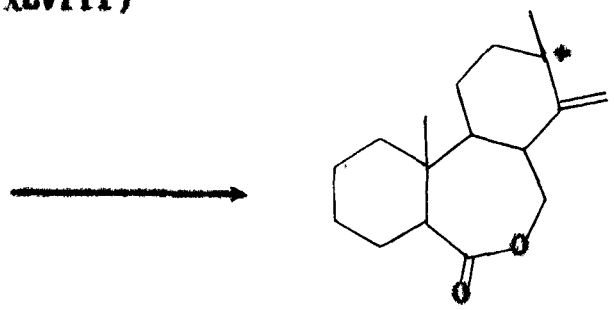
(CCXLVIII') R,H ; R', C_8H_{17}
(CCLXVIII') R,Cl; R', C_8H_{17}
(CCLV') R,OAc; R', $C_{10}H_{21}$

The subtle reason for the above difference in the peak number and their relative intensity in these isomeric lactones is not clearly understood and any attempt to rationalise this observation at this stage should be considered with reservation.

One of the possible explanations for the formation of the fragment ions m/e 262 and 261 from (CCXLVIII) may be shown according to the following scheme.



(CCXLVIII) m/e 262



m/e 261

However, this mechanism cannot be used for the formation of the ions m/e 260-262 from (CCLXVIII) and m/e 288-290 from (CCLV).

m/e 247

This fragment ion m/e 247 is usually a hydrocarbon species ($C_{18}H_{31}$) and is almost always present in the mass spectra of steroidal compounds. In the present case it is reasonable to suggest that its precursor may the ion m/e 262; the latter by the loss of a methyl group will produce this fragment ion.

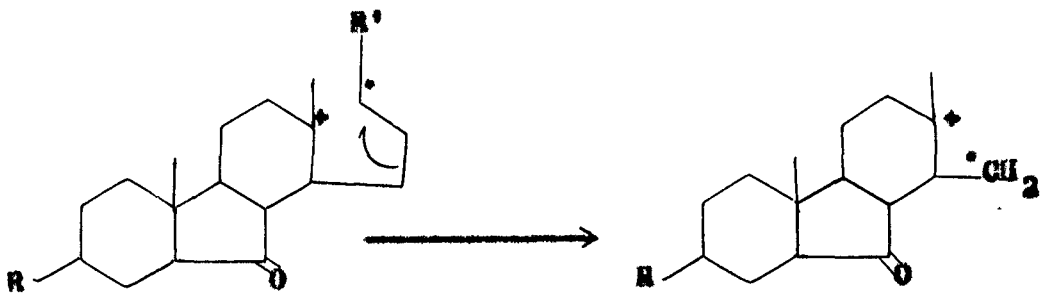
m/e 232

The fragment ion m/e 232 can be shown to arise from the B-norketonic species d (m/e 372) by a sequence of reactions already suggested for steroidal 6-ketones[88]. Analogous peaks for (CCLXVIII) and (CCLV) were observed at m/e 266/268 and m/e 290, respectively (Scheme-31).

m/e 218

The genesis of the ion m/e 218 from d can be shown according to Scheme-32. This proposal finds support from analogous peaks in (CCLXVIII, m/e 252/254) and (CCLV, m/e 276).

Scheme - 31



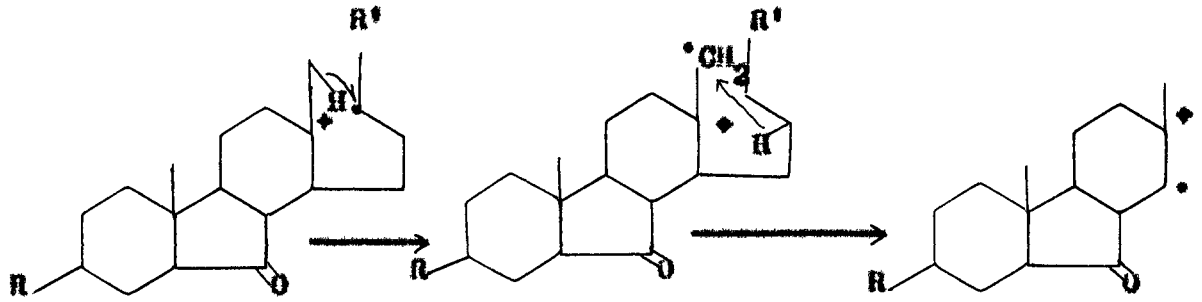
(d)

R, H/Cl/OAc; R', $C_8H_{17}/C_{10}H_{21}$

(i)

m/e 232; R,H m/e 290; R,OAc
m/e 266/268; R, Cl

Scheme - 32



(d)

m/e 218; R,H
m/e 252/254; R,Cl
m/e 276; R, OAc

The mass spectrum of 3β-chloro-7-oxa-B-homo-5α-cholestan-6-one (CCLXVIII) (Fig. 3) gave molecular ion peaks at m/e 436/438 (3:1, $C_{27}H_{45}O_2Cl$) along with other salient peaks at m/e 421/423 ($M-CH_3$), 418/420 ($M-H_2O$), 406/408 ($M-CH_2O$), 401 (M-Cl), 400 (M-HCl), 391/393 (406/408-CH_3), 351/353 (M-85, C_6H_{13}), 334, 323/325 ($M-C_8H_{17}$, side chain), 304, 293/295, 266/268, 262, 261, 260, 252/254, 247 and lower mass peaks.

The mass spectral values for 3β-chloro-6-oxa-B-homo-5α-cholestan-7-one (CCXLIII)(Fig. 4) are recorded below for the purpose of comparison. M 436/438 (3:1, $C_{27}H_{45}O_2Cl$), 421/423, 418/420, 408/410 (M-CO), 401 (M-Cl), 400 (M-HCl), 373 (401-CO), 372 (400-CO), 323/325 ($M-C_8H_{17}$), 318 (base peak, $C_{22}H_{38}O$), 317, 262, 247, 245 and lower mass peaks.

Fig.-4

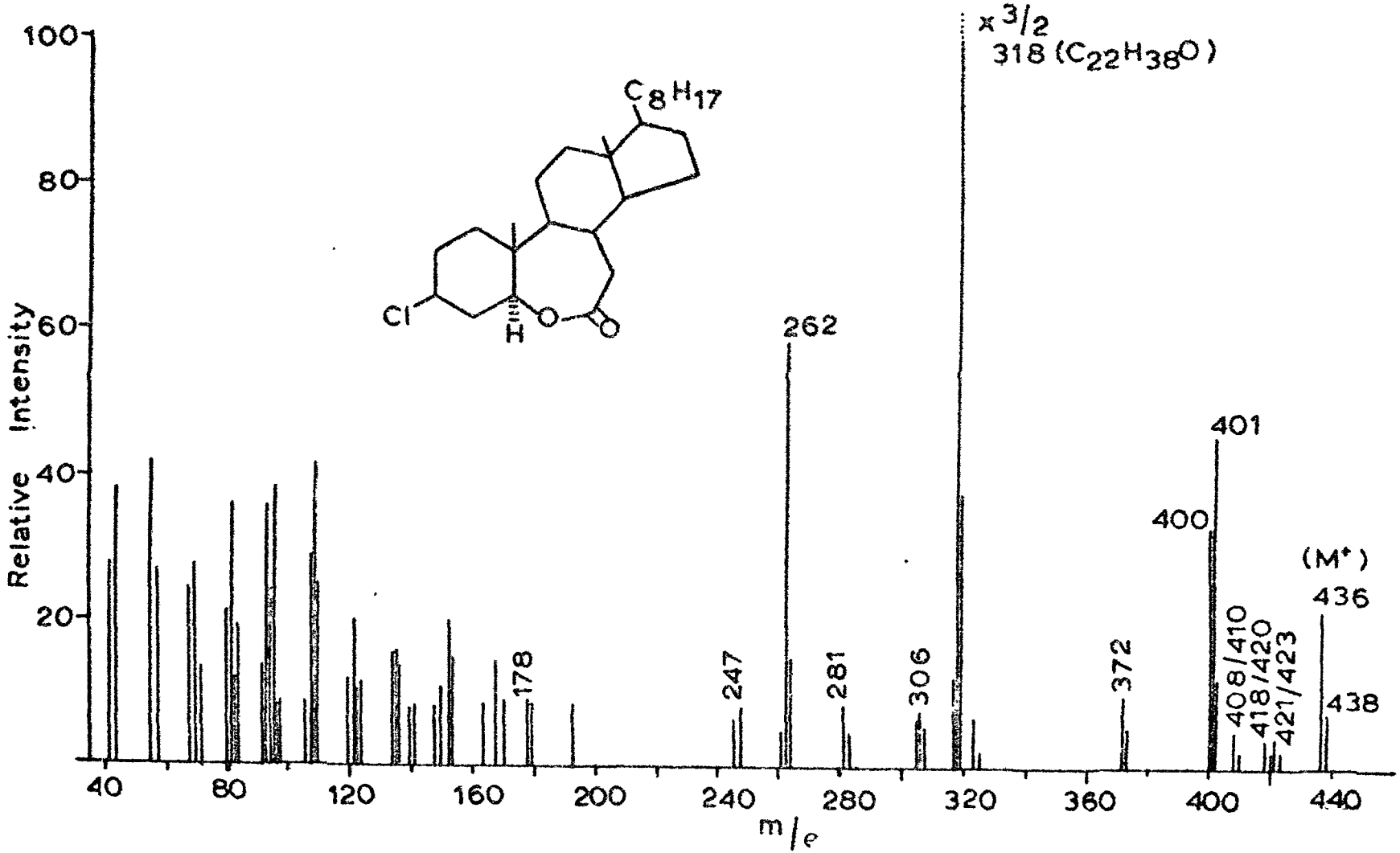

100
80
60
40
20
Relative Intensity
40
80
120
160
200
240
280
320
360
400
440
m/e
x 3/2
318 (C22H38O)
C8H17
Cl
H
O
O
262
401
400
(M+)
436
438
178
247
281
306
372
408/410
418/420
421/423

Fig.-4

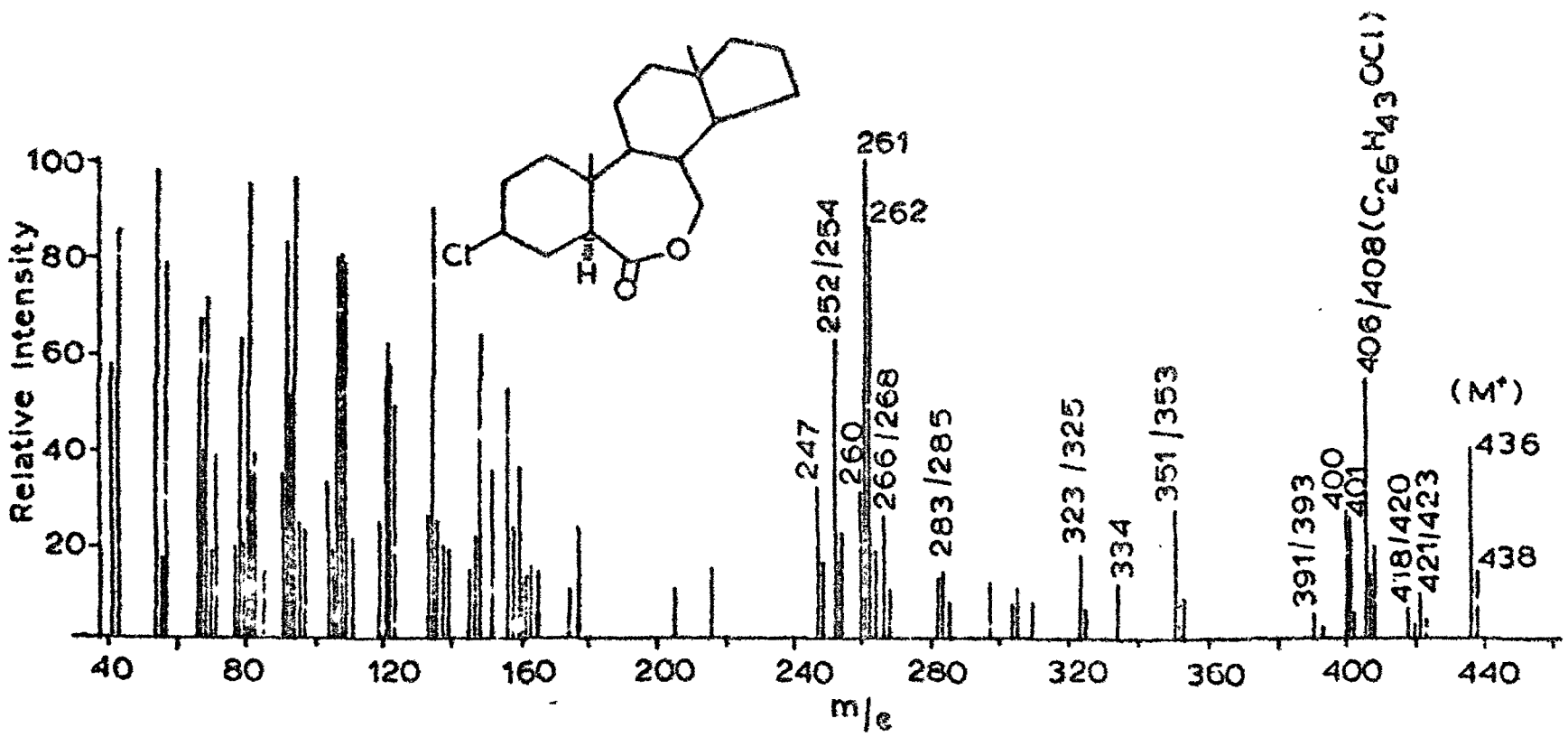
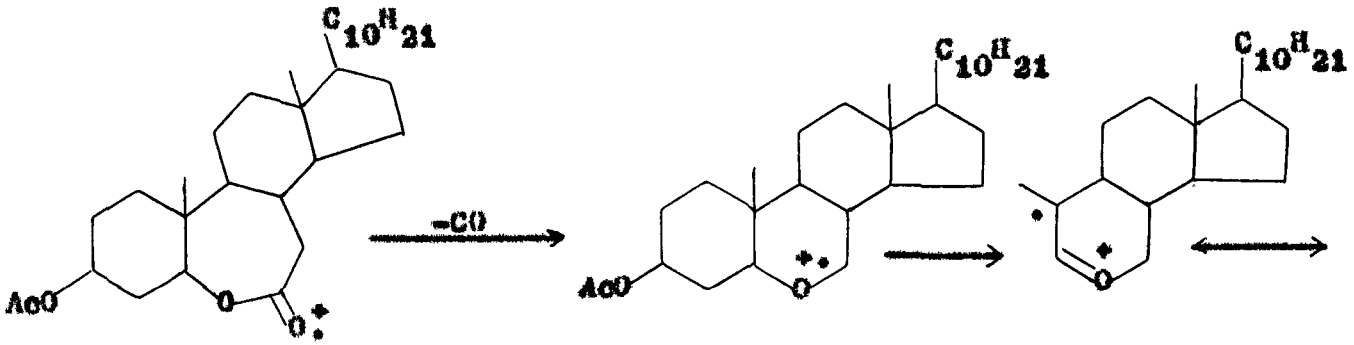

Relative Intensity
100
80
60
40
20
40
80
120
160
200
240
280
320
360
400
440
m/e
Cl
O
O
261
262
252/254
247
260
266/268
283/285
323/325
334
351/353
391/393
400
401
406/408(C26H43OCl)
418/420
421/423
(M+)
436
438

Fig.-3

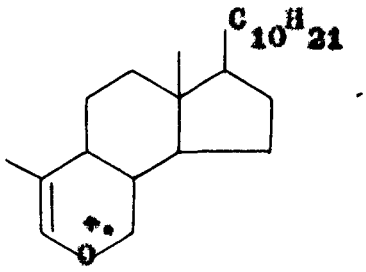
A comparison of the two spectra (Figs. 3 and 4) with previous ones (Figs. 1 and 2) clearly showsthat there is a striking similarity between the lactones (CCXLVIII) and (CCLXVIII) on the one hand and (XXXV) and (CCXLIII) on the other. The spectrum of (CCLXVIII) can conveniently be related with that of (CCXLVIII). As expected of (CCLXVIII), the loss of chlorine and HCl from the molecular ion occurred. As the important ions arising from (CCLXVIII) can be correlated with the ones obtained from (CCXLVIII), no attempt is being made to duplicate the interpretation already described.

The mass spectrum of 6-oxa-B-homo-7-oxo-3α-β-sitostanyl acetate (CCLVI)(Fig. 5) gave the molecular ion peak at m/e 488 ($C_{31}H_{52}O_4$) followed by other significant peaks at m/e 473 (M-CH_3), 470 (M-H_2O), 460 (M-CO), 446 (M-CH_2CO), 445 (M-CH_3,CO), 431(446-CH_3), 429 (M-AcOH), 413 (428-CH_3), 410 (428-H_2O), 400 (428-CO), 385 (400-CH_3), 382 (400-H_2O), 346 (base peak, $C_{24}H_{42}O$), 345 (346-H), 331 (346-CH_3), 290, 287 (428-side chain, $C_{10}H_{21}$), 275 (290-CH_3) 260, 259, 247, 245, 191, 178 and other lower mass peaks. A comparison of the spectrum of (CCLVI) with that of (XXXV) revealed a close similarity between the two. It was gratifying to note that, as expected the spectrum of (CCLVI) gave the base peak at m/e 346 ($C_{24}H_{42}O$) thus very convincingly supported the mechanism proposed for the genesis of the fragment ion m/e 318 ($C_{22}H_{38}O$) from (XXXV)(Scheme-24).

As postulated in Scheme-24 the fragmentation of the 6-oxalactones (XXXV, CCXLIII, CCXLIV) was shown to involve the loss of CO from the molecular ions, followed by the loss of ring A, thus leaving intact rings B,C,D and the side chain. A similar fragmentation with (CCLVI) resulted in the formation of analogous peak at m/e 346.



(CCLVI)



m/e 346 ($C_{24}H_{42}O$)

However, there is an interesting difference in the spectrum of (CCLVI) as compared with that of (XXXV). Whereas (XXXV) did not show the loss of a molecule of ketone (mass unit 42), the same cannot be said with equal degree of certainty about (CCLVI).

Fig.-5

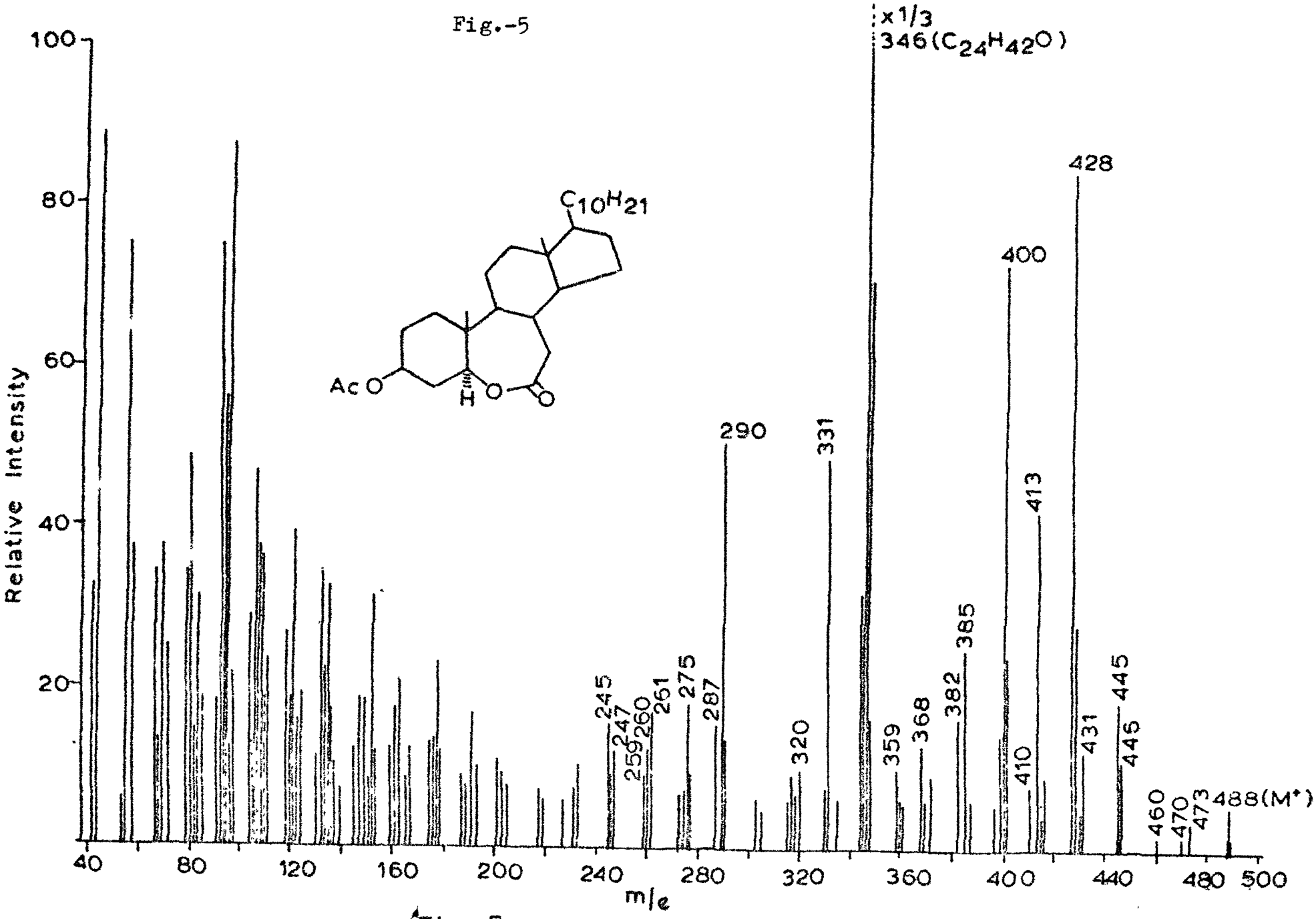

x1/3
346($C_{24}H_{42}O$)
$C_{10}H_{21}$
Ac O
H
O
O
Relative Intensity
100
80
60
40
20
40
80
120
160
200
240
280
320
360
400
440
480
500
m/e
428
400
413
290
331
385
382
368
359
320
445
446
431
410
245
247
259
260
261
275
287
460
470
473
488(M⁺)

Fig.- 5

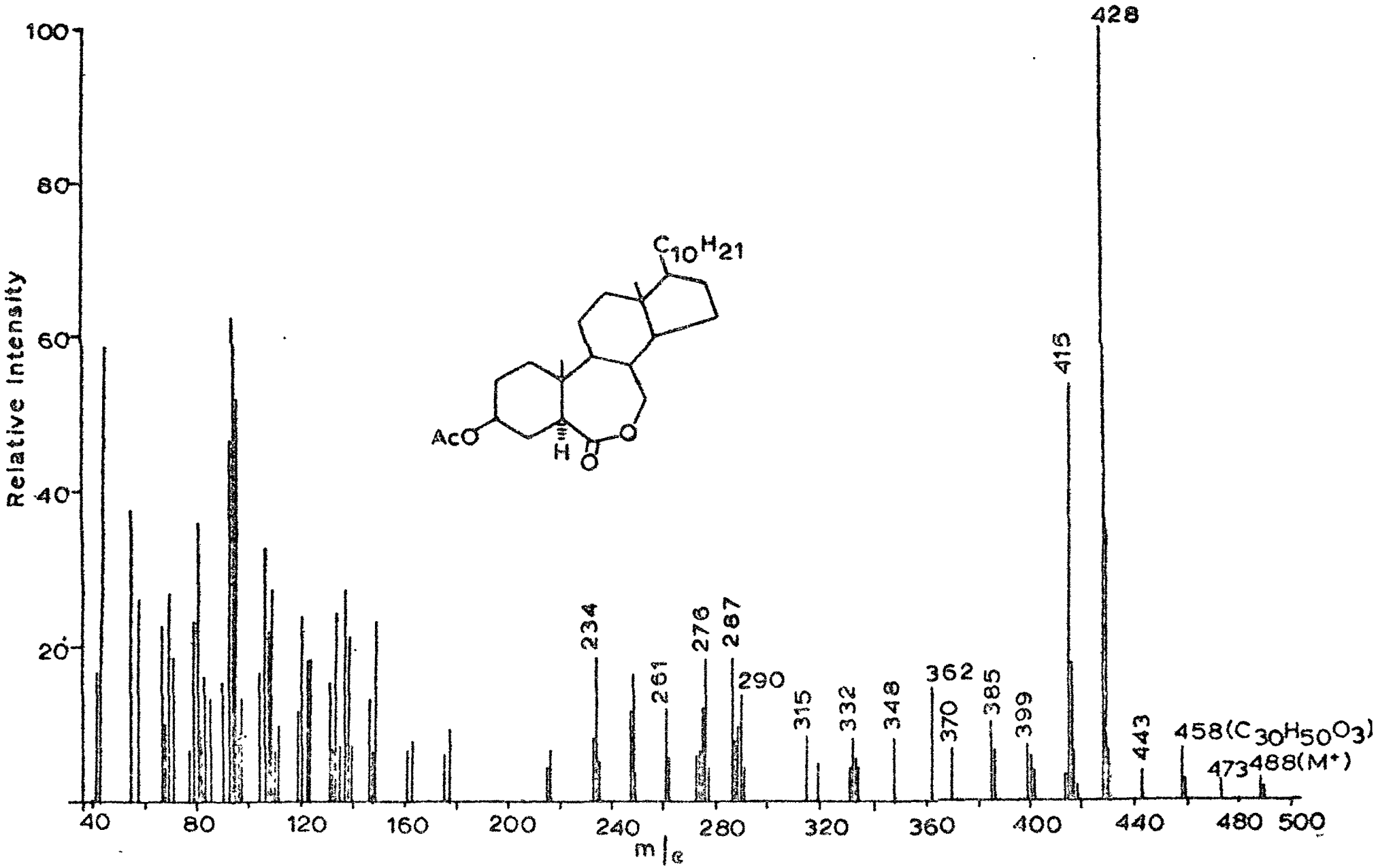

Relative Intensity
100
80
60
40
20
40
80
120
160
200
240
280
320
360
400
440
480
500
m/e
428
415
234
261
276
287
290
315
332
348
362
370
385
399
443
458(C30H50O3)
473
488(M+)
C10H21
AcO
H
O
O

Fig.-6

There is a reasonably acceptable peak at m/e 446 which is compatible with the loss of a ketene molecule from the molecular ion. As expected the loss of acetic acid from the molecular ion is very much in evidence.

The mass spectrum of the isomeric lactone, 7-oxa-8-homo-6-oxo-5α-β-sitostanyl acetate (CCLV)(Fig. 6) gave the molecular ion peak at m/e 488 ($C_{31}H_{52}O_4$) along with other significant peaks at m/e 473 (M-CH_3), 458 (M-CH_2O), 443 (458-CH_3), 428 (M-AcOH), 415 (443-CO), 400 (428-CO), 399, 385 (400-CH_3), 370, 362, 332 (362-CH_2O), 290, 299, 289, 287 (428-side chain), 276, 275, 274, 261, 248 (276-CO) and lower mass peaks.

The mass spectrum of (CCLV) is comparable with those of the other 7-oxalactones (CCXLVIII) and (CCLXVIII) and is distinct from its isomer (CCLVI).

Even without going through the fragmentation details of the two series of lactones it is possible to arrive at useful conclusions regarding the application of mass spectrometry in sensing the difference between the two groups.

(a) The 6-oxalactones (XXXV),(CCXLIII) and (CCLVI) split off CO from their respective molecular ions with subsequent loss of ring A to produce very distinct ion m/e 318 from (XXXV) and (CCXLIII) and the analogous ion m/e 346 from (CCLVI).

(b) The isomeric 7-oxalactones (CCXLVIII), (CCLXVIII) and (CCLV) on the other hand behave entirely different from their 6-oxa analogues. All the 7-oxalactones studied presently split off CH_2O (formaldehyde) from their respective molecular ions; interestingly no loss of CO was evident from the molecular ions.

(c) Both the 6-oxa and 7-oxalactones gave prominent peaks at m/e 262 in the cholestane series and at m/e 290 in β-sitostane series which shows that these ions are composed of C7a, rings C,D and the side chain. However, 7-oxalactones gave additional peaks at m/e 261, 260 (CCXLVIII and CCLXVIII) and the analogous peaks at 289 and 288 in the case of (CCLV).

(d) The 7-oxalactones show the loss of C_3, C_4, C_5 and C_6 to give fragment ion peaks at 334 (CCXLVIII and CCLXVIII) and analogous peak at 362 from (CCLV).

From the foregoing discussion it can safely be claimed that mass spectrometry offers an excellent means of differentiating between 6-oxa and 7-oxa ϵ-lactones in the cholestane and β-sitostane series.

EXPERIMENTAL

PART - I

All melting points are uncorrected. I.r. spectra were obtained with a Perkin-Elmer spectrophotometer and U.v. spectra with a Beckman DK_2 spectrophotometer. N.m.r. spectra were run in a Varian A60 instrument with $SiMe_4$ as the internal standard. Mass spectra were measured in an AEI MS 9 mass spectrometer using a direct insertion sample inlet system. Thin layer chromatographic plates were coated with silica gel and sprayed with a 20% aqueous solution of perchloric acid. Light petroleum refers to a fraction of b.p. 60-80°. N.m.r. values are given in ppm (s, singlet; d, doublet; dist.d, distorted doublet; dd, double of a doublet; br, broad; m, multiplet; a, peak disappears on addition of D_2O). I.r. values are given in cm^{-1} (s, strong; sh, shoulder, m, medium; w, weak; br, broad).

β-Sitosteryl acetate

A mixture of β-sitosterol (100 g), purified pyridine (150 ml) and freshly distilled acetic anhydride (100 ml) was heated on a steam bath for 2 hours. The light-brown solution was poured into crushed ice-water mixture with stirring. A white precipitate thus obtained was filtered under suction, washed with water (until free from pyridine) and air dried. The crude acetate was recrystallized from ethanol to give the pure product (90 g), m.p. 120°.

6-Nitro-β-sitosteryl acetate

β-Sitosteryl acetate (10 g) was covered with nitric acid (d, 1.52; 250 ml) and sodium nitrite (10 g) was gradually added over a period of 1 hour with continuous stirring. Slight external cooling was also effected during the course of the reaction and the stirring continued for additional 2 hours. A pale yellow spongy mass separated on the surface of the mixture. The mixture was diluted with cold water (200 ml) when a green coloured solution with yellow precipitate was obtained. The precipitate was separated by filtration and dissolved in ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%)(until the washing become pink), water and dried over anhydrous Na_2SO_4. Removal of the desiccant and solvent provided an oil which crystallized from ethanol (6.5 g), m.p. 79°.

6-Oxo-5α-β-sitostanyl acetate (CCLII)

6-Nitro-β-sitosteryl acetate (12 g) was dissolved in glacial acetic acid (500 ml) and zinc powder (24 g) added in small portions with shaking. The suspension was heated under reflux for 4 hours and water (25 ml) was added during the course of reaction. The hot solution was filtered, cooled to room temperature and diluted with large excess of water. The precipitate thus obtained was extracted with ether. The ethereal solution was washed with water,

$NaHCO_3$ solution (10%), water and dried over anhydrous Na_2SO_4. Removal of the desiccant and solvent gave an oil which crystallized from ethanol to provide the desired ketone (CCLII)(8.0 g), m.p. 120-21°; max (Nujol) 1740s (CH_3-COO), 1710s (C=O) and 1240 cm^{-1} (acetate).

The Baeyer-Villiger oxidation of 6-oxo-5α-β-sitostanyl acetate (CCLII); 7-Oxa-B-homo-6-oxo-5α-β-sitostanyl acetate (CCLV) and 6-oxa-B-homo-7-oxo-5α-β-sitostanyl acetate (CCLVI)

To a solution of 6-oxo-5α-β-sitostanyl acetate (CCLII) (2 g) in chloroform (30 ml) was added a chloroform solution of perbenzoic acid (1.1 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate as catalyst, and the reaction mixture was allowed to stand at room temperature for a week. (The progress of the reaction was checked by t.l.c.). The solvent was removed under reduced pressure and the residue extracted with ether. The ethereal solution was washed successively with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the desiccant and solvent provided a residue (ca. 2.0 g) which was chromatographed over silica gel (40 g) (each fraction of about 25 ml was collected. Elution with light petroleum-ether (10:1) gave the unreacted ketone (CCLII) (500 mg), m.p. and m.m.p. 120°. Elution with light petroleum-ether (6:1) afforded (CCLV), crystallized from light petroleum as shining needles (380 mg),

m.p. 130-31° (Found: C, 76.37; H, 10.56. $C_{31}H_{52}O_4$ requires C, 76.23; H, 10.65%). ν_{max} (Nujol) 1740s, 1715s, 1250s, 1210 and 1040 cm^{-1}; δ ($CDCl_3$) 4.66br (C3-αH, $W_{\frac{1}{2}}$ 14 Hz), 4.08br,s (C7a-βH), 3.98d (C7a-αH, J 3.5 Hz), 2.91dd (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 2.01s (CH_3COO), 0.9, 0.8 and 0.7 (6 methyl protons). Further elution with light petroleum-ether (5:1) furnished (CCLVI), crystallized from light petroleum (365 mg), m.p. 163-64° (Found: C, 76.33; H, 10.60. $C_{31}H_{52}O_4$ requires C, 76.23; H, 10.65%). ν_{max} (Nujol) 1740s, 1720s, 1240s and 1040 cm^{-1}; δ ($CDCl_3$) 4.75br (C3-αH, 14 Hz), 4.29dd (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5.5 Hz), 2.5br,s (C7a-βH), 2.43d (C7a-αH, J 3.5 Hz), 2.03s (CH_3COO), 0.9, 0.8 and 0.7 (6 methyl protons).

3β-Acetoxycholest-5-ene

A mixture of cholesterol (100 g), purified pyridine (150 ml) and freshly distilled acetic anhydride (100 ml) was heated on a steam bath for 2 hours. The working procedure, as described earlier, provided the crude acetate which was crystallized from acetone as fine needles (94.0 g), m.p. 115-16° (reported[74] m.p. 116°).

3β-Acetoxy-6-nitrocholest-5-ene

3β-Acetoxycholest-5-ene (10 g) was covered with nitric acid (d, 1.52; 250 ml) and sodium nitrite (10 g) was gradually added

over a period of 1 hour with continuous stirring. Slight cooling was also affected during the course of reaction and stirring was continued for additionnl 2 hours, when a yellow spongy mass separated on the surface of the mixture. The mixture was diluted with cold water (200 ml) when a green coloured solution was obtained. The whole mass was extracted with ether and worked up in the usual manner. Removal of the solvent provided the nitro-compound as an oil which was crystallized from methanol (6.5 g), m.p. 103° (reported[89] m.p. 102-4°).

3β-Acetoxy-5α-cholestan-6-one (XXXIV)

3β-Acetoxy-6-nitrocholest-5-ene (12 g) was dissolved in glacial acetic acid (500 ml) by warming the mixture on water bath and zinc powder (20 g) was added in small portions with shaking. The suspension was heated under reflux for 4 hours and water (25 ml) was added now and then during the course of reaction. The hot solution was filtered, cooled to room temperature and diluted with a large excess of cold water. The precipitate thus obtained was taken in ether and the ethereal solution was worked up in usual fashion. Removal of the solvent provided an oil which was crystallized from methanol to furnish (XXXIV)(8.5 g), m.p. 128° (reported[90] m.p. 127-28°).

The Baeyer-Villiger oxidation of 3β-acetoxy-5-ᾱ-cholestan-6-one (XXXIV); 3β-Acetoxy-7-oxa-8-homo-5-ᾱ-cholestan-6-one (CCLVII) and 3β-acetoxy-6-oxa-8-homo-5-ᾱ-cholestan-7-one (XXXVI)

Reaction of 3β-acetoxy-5-ᾱ-cholestan-6-one (XXXIV) (2 g) with perbenzoic acid (1.1 mole equivalent) was performed in the usual manner to provide a semi solid material which was chromatographed over silica gel. Elution with light petroleum-ether (10:1) gave the unreacted (XXXIV)(450 mg), m.p.^{90} and m.m.p. 127°. Elution with light petroleum-ether (7:1) afforded (CCLVII), crystallized from light petroleum as needles (460 mg), m.p. 181° (Found: C, 75.75; H, 10.33. $C_{29}H_{48}O_4$ requires C, 75.65; H, 10.43%). ν max (Nujol) 1740s, 1715s, 1250s, 1205 and 1035 cm^{-1}; δ($CDCl_3$) 4.66br (C3-ᾱ$\underline{H}$, $W_{\frac{1}{2}}$ 14 Hz), 4.1br,s (C7a-β$\underline{H}$), 4.0d (C7a-ᾱ$\underline{H}$, J 3.5 Hz), 2.92dd (C5-ᾱ$\underline{H}$, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 2.03s ($C\underline{H}_3COO$), 0.9, 0.8 and 0.7 (3 methyl protons). Further elution with light petroleum-ether (6:1) gave (XXXVI), crystallized from light petroleum (455 mg), m.p. 174° (reported[13] m.p. 162-63°)(Found: C, 75.51; H, 10.47. Calcd. for $C_{29}H_{48}O_4$ C, 75.65; H, 10.43%). ν max (Nujol) 1740s, 1718s, 1245s and 1035 cm^{-1}; δ($CDCl_3$) 4.75br (C3-ᾱ$\underline{H}$, $W_{\frac{1}{2}}$ 14 Hz), 4.29dd (C5-ᾱ$\underline{H}$, $J_{a,a}$ 10 Hz; $J_{a,e}$ 5.5 Hz), 2.5br,s (C7a-β$\underline{H}$), 2.42d (C7a-ᾱ$\underline{H}$, 3.5 Hz), 2.03s ($C\underline{H}_3COO$), 0.9, 0.8 and 0.7 (5 methyl protons).

3β-Hydroxy-7-oxa-B-homo-5α-cholestan-6-one (CCLVIII)

A solution of (CCLVII)(250 mg) in 50 ml methanolic NaOH (2%) was heated under reflux for 1 hour. The solution was acidified with dil. HCl and poured into water. The precipitate thus obtained was extracted with ether and successively washed with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the solvent provided the hydroxy lactone (CCLVIII), crystallized from light petroleum (210 mg), m.p. 124° (Found: C, 77.45; H, 10.90. $C_{27}H_{46}O_3$ requires C, 77.51; H, 11.0%). ν max (Nujol) 3430br, 1715s, 1195 and 1060 cm^{-1}.

3β-Hydroxy-6-oxa-B-homo-5α-cholestan-7-one (CCLIX)

The acetate function in (XXXVI) (250 mg) was hydrolysed in the manner described for (CCLVII). Subsequent work up and removal of the solvent gave (CCLIX), crystallised from light petroleum (200 mg), m.p. 202° (reported[13] m.p. 139-41°) (Found: C, 77.42; H, 11.07. Calcd. for $C_{27}H_{46}O_3$ C, 77.51; H, 11.0%). ν max (Nujol) 3300br, 1718s, 1225 and 1025 cm^{-1}.

3β-Hydroxy-5α-cholestan-6-one (CCLIII)

3β-Acetoxy-5α-cholestan-6-one (XXXIV) (5 g) was dissolved in 400 ml methanolic NaOH (2%) and heated under reflux for 1 hour.

The excess of the solvent was removed by distillation under reduced pressure. The residue was poured into water, acidified with dil. HCl and the precipitate thus obtained was extracted with ether. The usual work up of the ethereal solution and removal of the the solvent provided (CCLIII), crystallized from methanol (3.8 g), m.p. 143° (reported[90] m.p. 142°- 43°),

The Baeyer-Villiger oxidation of 3β-hydroxy-5α-cholestan-6-one (CCLIII); 3β-Hydroxy-7-oxa-8-homo-5α-cholestan-6-one (CCLVIII) and 3β-hydroxy-6-oxa-8-homo-5α-cholestan-7-one(CCLIX)

— 3β-Hydroxy-5α-cholestan-6-one (CCLIII) (2 g) was treated with perbenzoic acid (1.1 mole equivalent) in the usual manner to provide a residue which was chromatographed over silica gel. Elution with chloroform-benzene (8:1) gave the unreacted ketone (CCLIII)(345 mg), m.p.[90] and m.m.p. 143°. Elution with chloroform furnished (CCLVIII)(380 mg), m.p. and m.m.p. 124°. Continued elution with the same solvent furnished (CCLIX) (370 mg), m.p. and m.m.p. 202°.

Acetylation of (CCLVIII)

A mixture of 3β-hydroxy-7-oxa-8-homo-5α-cholestan-6-one (CCLVIII)(100 mg), purified pyridine (0.6 ml) and freshly distilled acetic anhydride (0.4 ml) was allowed to stand at room temperature for 48 hours. The reaction mixture was poured into water and

precipitate thus obtained was extracted with ether. The ethereal solution was washed with water, dil. HCl (until free from pyridine), water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the solvent provided (CCLVII) (85 mg), m.p. and m.m.p. 181°.

Acetylation of (CCLIX)

3β-Hydroxy-6-oxa-B-homo-5α-cholestan-7-one (CCLIX) (100 mg) was acetylated in the manner described above for (CCLVIII) to afford (CXXXVI)(90 mg), m.p. and m.m.p. 174°.

7-Oxa-B-homo-5α-cholestane-3,6-dione (CCLXII)

The hydroxy lactone (CCLVIII)(300 mg) was dissolved in acetone (40 ml) and cooled below 10°C in ice bath. Jones' reagent (0.5 ml) was added slowly with continuous stirring. Water (40 ml) was added to it and the precipitate thus obtained extracted with ether. The ethereal solution was washed with water and dried over anhydrous Na_2SO_4. Removal of the solvent gave (CCLXII), crystallised from light petroleum as needles (230 mg), m.p. 195° (Found; C, 77.80; H, 10.59. $C_{27}H_{44}O_3$ requires C, 77.88; H, 10.57%). ν max. (Nujol) 1725s, 1720s, 1230, 1185 and 1085 cm^{-1}.

Attempted base-catalysed hydrolysis of the lactones (CCLXII), (CCLVII) and (XXXVI)

(i) A solution of (CCLXII)(200 mg) in 40 ml of methanolic NaOH (5%) was heated under reflux for 2 hours. The solution was diluted with water, carefully acidified with dilute acetic acid, and extracted with ether. The ether extracts were washed with water, dried over anhydrous Na_2SO_4 and evaporated. Immediate t.l.c. of the residue showed two spots of about equal intensity (one lactone CCLXII and the other probably the seco acid CCLXIII). However, on standing the ethereal solution of the mixture at room temperature for 3-4 hours, relactonization occurred as was evident from a single spot (t.l.c.) identical with the lactone (CCLXXII) (170 mg), m.p. and m.m.p. 195°.

(ii) The above reaction was repeated and efforts were made to separate them by chromatography over silica gel but (CCLXIII) relactonized during the passage through silica gel as elution afforded only the lactone (CCLXII), m.p. and m.m.p. 195°.

The aforesaid observations were noted for the lactones (CCLVII) and (XXXVI) also, in which relactonized products (CCLVIII) and (CCLIX) were obtained from (CCLVII) and (XXXVI), respectively.

6-Oxa-B-homo-5α-cholestane-3,7-dione (CCLXIV)

The hydroxy lactone (CCLIX)(300 mg) was treated with Jones' reagent in the manner described for (CCLVIII). Subsequent work up afforded (CCLXIV), crystallized from light petroleum (260 mg), m.p. 191° (Found: C, 77.93; H, 10.46. $C_{27}H_{44}O_3$ requires C, 77.88; H, 10.57%). ν max. (Nujol) 1722s, 1720s, 1275 and 1040 cm^{-1}.

3-Oxo-5,6-secocholest-4-en-6-oic acid (CCLXVI)

A solution of (CCLXIV)(200 mg) in 40 ml of methanolic NaOH (5%) was heated under reflux for 2 hours. The excess of methanol was removed under reduced pressure and the residue poured into water, acidified with HCl and extracted with ether. The usual work up provided (CCLXVI) as a noncrystallizable oil (170 mg) (Found: C, 77.79; H, 10.50. $C_{27}H_{44}O_3$ requires C, 77.88; H, 10.57%). λ max (EtOH) 230 nm (ϵ 10000); ν max (Nujol) 3550-3200br, 1725s, 1675s, 1610 and 1180 cm^{-1}.

Methyl 3-oxo-5,6-secocholest-4-en-6-onte (CCLXVII)

An ethereal solution of (CCLXVI)(120 mg) was treated with an excess of an ethereal solution of diazomethane and allowed to stand for 10 minutes in the cold. Excess of diazomethane was decomposed with a little amount of acetic acid. The usual work up

and removal of the solvent provided the desired methyl ester (CCLXVII) as an oil (110 mg) (Found: C, 78.16; H, 10.68. $C_{29}H_{46}O_3$ requires C, 78.13; H, 10.69%). λ max (EtOH) 230 nm (ε 9860); νmax (Nujol) 1735s, 1680s and 1190 cm^{-1}; δ ($CDCl_3$) 5.75d (C3-$\underline{H}$, J 10 Hz), 5.68d (C4-$\underline{H}$, J 10 Hz), 3.6s ($COOC\underline{H}_3$), 2.51mc (C2-$\underline{H}_2$ and C7-$\underline{H}_2$), 1.2, 0.9, 0.8 and 0.66 (5 methyl protons).

3β-Chlorocholest-5-ene

Freshly purified thionyl chloride (75 ml) was added gradually to cholesterol (100 g) at room temperature. A vigorous reaction ensued with the evolution of gaseous products. When the reaction slackened the mixture was gently heated at a temperature of 50-60°C on a water bath for 1 hour, and then poured onto crushed ice with stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-cooled water and air dried. Recrystallization from acetone gave 3β-chlorocholest-5-ene (92.0 g), m.p. 95-96° (reported[91] m.p. 96-97°).

Cholest-5-ene

3β-Chlorocholest-5-ene (10 g) was dissolved in warm amyl alcohol (230 ml) and sodium metal (20 g) was added to the solution with continuous stirring over a period of 8 hours. The reaction mixture was warmed occasionally. When all the sodium metal was

dissolved, the reaction mixture was poured into 50% HCl (500 ml) and then allowed to stand overnight. A white crystalline solid thus obtained was filtered under suction and washed thoroughly with water and air dried. The crude product was crystallised from acetone to provide, the desired compound as cubes (8.5 g), m.p. 94° (reported[99] m.p. 95°).

6-Nitrocholest-5-ene

A suspension of finely powdered cholest-5-ene (6 g) in glacial acetic acid (50 ml) was vigorously stirred at room temperature and treated with nitric acid (d, 1.52; 15 ml), followed by the addition of sodium nitrite (3 g) over a period of 1 hour. The reaction mixture was poured into cold water and the yellow product thus obtained was extracted with ether. Usual work up and removal of the solvent provided the desired compound as an oil which was crystallized from ethanol as leaflets (4.5 g), m.p. 119-20° (reported[92] m.p. 120-21°).

5 -Cholestan-6-one (XXXIII)

6-Nitrocholest-5-ene (12 g) was dissolved in warm glacial acetic acid (400 ml) and zinc powder (24 g) was gradually added with shaking. The mixture was heated under reflux for 4 hours and water(20 ml) added during the course of reaction. Zinc powder

was removed by filtration and the filtrate was diluted with water. 5α-Cholestan-6-one (XXXIII) crystallized out as thin plates, and was recrystallized from ethanol (3.5 g), m.p. 97-98° (reported[93] m.p. 98-100°).

The Baeyer-Villiger oxidation of 5α-cholestan-6-one (XXXIII); 7-Oxa-8-homo-5α-cholestan-6-one (CCXLVIII) and 6-oxa-8-homo-5α-cholestan-7-one (XXXV)

Reaction of 5α-cholestan-6-one (XXXIII) (2 g) with perbenzoic acid (1.1 mole equivalent) in the usual manner gave a solid residue which was chromatographed over silica gel. Elution with light petroleum-ether (14:1) gave the unreacted ketone (XXXIII)(300 mg), m.p.[93] and m.m.p. 98-99°. Elution with light petroleum-ether (11:1) provided (CCXLVIII), crystallized from light petroleum as needles (250 mg), m.p. 126°(Found: C, 80.45; H, 11.39. $C_{27}H_{46}O_2$ requires C, 80.59; H, 11.44%). ν max (Nujol) 1722s, 1185, 1135 and 1080 cm^{-1}; δ ($CDCl_3$) 4.26br,s (C7a-βH), 4.16d (C7a-αH, J 3.5 Hz), 2.66dd (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons). Continued elution with the same solvent system furnished (XXXV) crystallized from light petroleum as needles (760 mg), m.p. 155° (reported[13] m.p. 143-44°) (Found: C, 80.77; H, 11.51. Calcd. for $C_{27}H_{46}O_2$ C, 80.39; H, 11.44%). νmax (Nujol) 1720s, 1275 and 1035 cm^{-1}; δ ($CDCl_3$) 4.16dd (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 5 Hz), 2.5br,s (C7a-βH), 2.41d (C7a-αH, J 3.5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons).

3β-Chloro-6-nitrocholest-5-ene

To a well stirred mixture of 3β-chlorocholest-5-ene (12 g), glacial acetic acid (60 ml) and nitric acid (d, 1.52; 25 ml) at temperature below 20°, was added sodium nitrite (6 g) gradually over a period of 2 hours. After the complete addition of sodium nitrite, the mixture was further stirred for about 1 hour. Ice-cooled water (200 ml) was added and the yellowish solid thus separated was filtered under suction and air dried. The product was crystallized from methanol as needles (8.0 g), m.p. 151-52° (reported[94] m.p. 153°).

3β-Chloro-5α-cholestan-6-one (CCXLIX)

To a solution of 3β-chloro-6-nitrocholest-5-ene (12 g) in hot glacial acetic acid (350 ml), zinc powder (24 g) was added gradually in small portions with shaking. The suspension was heated under reflux for 4 hours and water (24 ml) was added at regular intervals during the course of reaction. The hot solution was filtered and the filtrate was cooled to room temperature followed by dilution with a large excess of ice-cooled water. The organic material was extracted with ether. The usual work up and removal of the solvent furnished (CCXLIX) as an oil which was crystallized from methanol (8.5 g), m.p. 128-29° (reported[95] m.p. 129°).

The Baeyer-Villiger oxidation of 3β-chloro-5α-cholestan-6-one (CCXLIX); 3β-Chloro-7-oxa-8-homo-5α-cholestan-6-one (CCLXVIII) and 3β-chloro-6-oxa-8-homo-5α-cholestan-7-one (CCXLIII)

3β-Chloro-5α-cholestan-6-one (CCXLIX) (2 g) on treatment with perbenzoic acid (1.1 mole equivalent) in the usual fashion and subsequent work up gave a residue which was chromatographed over silica gel. Elution with light petroleum-ether (11:1) gave the unreacted ketone (CCXLIX)(540 mg), m.p.[95] and m.m.p. 120°. Elution with light petroleum-ether (8:1) furnished (CCLXVIII), crystallized from light petroleum as fine needles (350 mg), m.p. 145° (Found: C, 74.35; H, 10.25. $C_{27}H_{45}O_2Cl$ requires C, 74.22; H, 10.30%). νmax (Nujol) 1715s, 1195, 1130, 1085 and 735 cm^{-1}; δ($CDCl_3$) 4.09br,s (C7a-β<u>H</u>), 4.0d (C7a-α<u>H</u>, *J* 5 Hz), 3.70br (C3-α<u>H</u>, $w\frac{1}{2}$ 14 Hz), 2.85dd (C5-α<u>H</u>, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons). Further elution with light petroleum-ether (7:1) gave (CCXLIII), crystallized from light petroleum (345 mg), m.p. 185° (reported m.p.[69] 187-88°) (Found: C, 74.20; H, 10.34. Calcd. for $C_{27}H_{45}O_2Cl$ C, 74.22; H, 10.30%). νmax (Nujol) 1718s, 1280, 1045 and 740 cm^{-1}; δ($CDCl_3$) 4.21dd (C5-α<u>H</u>, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 3.66br (C3-α<u>H</u>, $w\frac{1}{2}$ 14 Hz), 2.5br,s (C7a-β<u>H</u>), 2.41d (C7a-α<u>H</u>, J 5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons).

Sodium-pentyl alcohol reduction of (CCLXVIII); 7-Oxa-B-homo-5∝-cholestan-6-one (CCXLVIII)

The chlorolactone (CCLXVIII) (200 mg) was dissolved in warm pentyl alcohol (10 ml) and to this solution was added sodium metal (1 g) in small portions with intermittent heating during 30 minutes. The solution was kept warm for an additional period of 2 hours. When all the metal had dissolved, the reaction mixture was poured into cold water, acidified with HCl and worked up in the usual manner. Removal of the solvent under reduced pressure and its column chromatography over silica gel afforded (CCXLVIII)(120 mg), m.p. and m.m.p. 126°.

Sodium-pentyl alcohol reduction of (CCXLIII); 6-Oxa-B-homo-5∝-cholestan-7-one (XXXV)

The chlorolactone (CCXLIII)(200 mg) was subjected to reduction in the manner described for (CCLXVIII). The usual work up followed by column chromatography over silica gel afforded (XXXV)(90 mg), m.p. and m.m.p. 155°.

3∝,5-Cyclo-5∝-cholestan-6-one (CCX)

A mixture of 3β-chloro-5∝-cholestan-6-one (CCXLIX) (5 g) and methanolic KOH (75 ml, containing 3.7 g of KOH) was heated under reflux for 1 hour. The reaction mixture was poured into

water and extracted with ether. The ethereal solution was washed successively with water, dil. HCl, water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the solvent gave the cycloketone (CCX) which was crystallized from methanol (3.5 g), m.p. 96-97° (reported[96] m.p. 97°).

3β-Bromo-5α-cholestan-6-one (CCL)

3α,5-Cyclo-5α-cholestan-6-one (CCX)(2 g) was heated under reflux with HBr (48%; 2.5 ml) in acetone (7.5 ml) for about 6 hours. Most of the solvent was removed under reduced pressure and the residue diluted with water (20 ml). A solid thus obtained was filtered and crystallized from methanol to give the bromoketone (CCL)(1.5 g), m.p. 126-27° (reported[97] m.p. 124°).

The Baeyer-Villiger oxidation of 3β-bromo-5α-cholestan-6-one(CCL); 3β-bromo-7-oxa-B-homo-5α-cholestan-6-one (CCLXIX) and 3β-bromo-6-oxa-B-homo-5α-cholestan-7-one (CCXLIV)

3β-Bromo-5α-cholestan-6-one (CCL)(2 g) was treated with perbenzoic acid (1.1 mole equivalent) in the usual manner to provide a residue which was chromatographed over silica gel. Elution with light petroleum-ether (12:1) gave the unreacted ketone (CCL)(620 mg), m.p.[97] and m.m.p. 124°. Elution with light petroleum-ether (8:1) afforded (CCLXIX), crystallized from light petroleum as fine needles (460 mg), m.p. 171° (Found: C, 67.42;

H, 9.32. $C_{27}H_{45}O_2Br$ requires C, 67.35; H, 9.35%). ν max (Nujol) 1712s, 1190, 1130, 1080 and 720 cm^{-1}; δ ($CDCl_3$) 4.1br,s (C7a-βH), 4.01d (C7a-αH, J 5 Hz), 3.68br (C3-αH, $W_{\frac{1}{2}}$ 14 Hz), 2.84dd (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons). Further elution with light petroleum-ether (7:1) furnished (CCXLIV), crystallized from light petroleum (375 mg), m.p. 183° (reported[69] m.p. 179°)(Found: C, 67.45; H, 9.36. Calcd. for $C_{27}H_{45}O_2Br$ C, 67.35; H, 9.35%). νmax (Nujol) 1715s, 1280, 1035 and 720 cm^{-1}; ($CDCl_3$) 4.2dd (C5-αH, $J_{a,a}$ 11 Hz; $J_{a,e}$ 5 Hz), 3.70br (C3-αH, $W_{\frac{1}{2}}$ 14 Hz), 2.51br,s (C7a-βH, 2.41d (C7a-αH, J 5 Hz), 0.9, 0.8 and 0.7 (5 methyl protons).

Sodium-pentyl alcohol reduction of (CCLXIX): 7-Oxa-B-homo-5α-cholestan-6-one (CCXLVIII)

The bromolactone (CCLXIX)(200 mg) was treated with sodium-pentyl alcohol in the manner as described earlier. The usual work up and column chromatography over silica gel furnished (CCXLVIII) (100 mg), m.p. and m.m.p. 126°.

Sodium-pentyl alcohol reduction of (CCXLIV): 6-Oxa-B-homo-5α-cholestan-7-one (XXXV)

The bromolactone (CCXLIV)(200 mg) was reduced with sodium-pentyl alcohol in the usual fashion. Usual work up of the reaction mixture, followed by column chromatography gave the lactone (XXXV) (85 mg), m.p. and m.m.p. 155°.

The Baeyer-Villiger oxidation of 3α,5-cyclo-5α-cholestan-6-one (CCX); 6-Oxa-3α,5-cyclo-6-homo-5α-cholestan-7-one (CCXLV)

Reaction of 3α,5-cyclo-5α-cholestan-6-one (CCX)(2 g) with perbenzoic acid (1.1 mole equivalent) in the usual manner gave a solid residue which was chromatographed over silica gel. Elution with light petroleum-ether (15:1) gave the unreacted ketone (CCX) (650 mg), m.p.[96] and m.m.p. 97°. Further elution with light petroleum-ether (9:1) provided (CCXLV), crystallized from light petroleum (900 mg), m.p.[69] and m.m.p. 126°.

3β-Acetoxy-5α-bromocholestan-6-one (XXXVIII)

To a cooled solution of 3β-acetoxy-5α-cholestan-6-one (XXXIV) (2 g) in acetic acid (5 ml) and ether (18 ml), bromine solution (1.1 g bromine in 15 ml acetic acid) was added gradually. Few drops of HBr (48%) were added to catalyse the reaction. The desired bromocompound (XXXVIII) thus obtained was filtered and recrystallized from chloroform-ether (1.2 g), m.p. 164° (reported[98] m.p. 162°).

3β-Acetoxycholest-4-en-6-one (CCLIV)

A solution of 3β-acetoxy-5α-bromocholestan-6-one (XXXVIII) (5 g) and purified pyridine (50 ml) was heated under reflux for

about 8 hours under anhydrous conditions. The reaction mixture was poured into ice-cooled water, acidified with HCl and extracted with ether. The usual work up and removal of the solvent provided an oil which was crystallised from methanol to give the ketone (CCLIV)(3.1 g), m.p. 110° (reported[98] m.p. 110°). λ max (EtOH) 238 nm (ϵ 6000); ν max (Nujol) 1740s, 1690s, 1635 and 1240 cm^{-1}; δ($CDCl_3$) 6.09 dist.d (C4-$\underline{H}$, major J 1.5 Hz), 5.35m (C3-$\underline{H}$, $W\frac{1}{2}$ 14 Hz), 2.5m (C7-$\underline{H}_2$), 2.05s ($C\underline{H}_3COO$), 1.0 (C10-CH_3), 0.7 (C13-CH_3), 0.9 and 0.8 (other methyl protons).

5α-Cholestane-3,6-dione (CCXV)

(i) A mixture of 3β-acetoxycholest-4-en-6-one (CCLIV) (2 g), conc. HCl (2 ml) and ethanol (50 ml) was heated under reflux for 1 hour. Half of the alcohol was removed under reduced pressure when the dione (CCXV) started crystallizing out. The solid was filtered under suction and recrystallized from ethanol (1.2 g), m.p. 168° (reported[98] m.p. 169°).

(ii) 3β-Hydroxy-5α-cholesten-6-one (CCLIII) (5 g) was dissolved in acetone (150 ml) and cooled below 10° in an ice bath. Jones' reagent (10 ml) was added slowly in 30 minutes with continuous stirring and the stirring continued for 1 hour. Water (200 ml) was added to the mixture and the solid material extracted with an excess of ether. The ethereal solution was washed with water

and dried over anhydrous Na_2SO_4. Removal of the solvent gave (CCXV) which was recrystallized from ethanol (1.7 g), m.p. and m.m.p. 168°.

The Baeyer-Villiger oxidation of 5α-cholestane-3,6-dione (CCXV): 3-Oxa-A-homo-5α-cholestane-4,6-dione (CCLXX)

5α-Cholestane-3,6-dione (CCXV)(2 g) was dissolved in chloroform (25 ml) and to this was added a solution of perbenzoic acid in chloroform (different concentrations) alongwith a few crystals of p-toluenesulphonic acid monohydrate as catalyst. The reaction mixture was kept at room temperature for 48 hours. The solvent was removed under reduced pressure and the residue extracted with ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. The removal of the solvent provided a residue (ca. 2.0 g) which was chromatographed over silica gel (40 g) (each fraction of 30 ml was collected). Elution with light petroleum alone and with benzene gave no material. Elution with benzene-ether (5:1) afforded the compound (CCLXX), crystallized from methanol (400 mg), m.p. 220° (Found: C, 77.90; H, 10.58. $C_{27}H_{44}O_3$ requires C, 77.88; H, 10.57%). ν max (Nujol) 1735s, 1700s and 1050 cm^{-1}; δ ($CDCl_3$) 4.27m (C2-$\underline{H}_2$), 3.15-2.3m (C4a-$\underline{H}_2$, C5-$\underline{H}$ and C7-$\underline{H}_2$), 1.21, 0.92, 0.86 and 0.68 (5 methyl protons).

6-Oxo-2,3-seco-2-hydroxycholestan-3-oic acid (CCLXXII)

A solution of the lactone (CCLXX)(100 mg) in 20 ml of methanolic NaOH (5%) was heated under reflux for 2 hours. The solution was poured into water, carefully acidified with HCl and extracted with ether. The usual work up and removal of the solvent provided the desired secoacid (CCLXXII) which was crystallized from methanol (80 mg), m.p. 188-90° (Found: C, 74.85; H, 10.45. $C_{27}H_{46}O_4$ requires C, 74.65; H, 10.59%). ν max (Nujol) 3300br, 1710s and 1700sh cm^{-1}.

Methyl-6-oxo-2,3-seco-2-hydroxycholestan-3-oate (CCLXXIII)

An ethereal solution of the secoacid (CCLXXII) (150 mg) was treated with an excess of ethereal solution of diazomethane and the mixture was allowed to stand for 10 minutes in the cold. Excess of diazomethane was decomposed with a little amount of acetic acid. The usual work up and removal of the solvent provided the desired methyl ester (CCLXXIII) (120 mg), m.p. 90° (Found: C, 75.20; H, 10.55. $C_{28}H_{48}O_4$ requires C, 75.0; H, 10.71%). νmax (Nujol) 3500br, 1720sh, 1710s and 1190 cm^{-1}; δ($CDCl_3$) 3.63s ($COOC\underline{H}_3$), 3.63m (C2-$\underline{H}_2$), 3.2-2.15m (C4-$\underline{H}_2$, C5-$\underline{H}$ and C7-$\underline{H}_2$), 2.1m ($O\underline{H}^a$), 1.23, 0.88, 0.8 and 0.6 (5 methyl protons).

Baeyer-Villiger oxidation of 3β-acetoxycholest-4-en-6-one (CCLIV); 4-hydroxycholesta-2,4-dien-6-one (CCLXXIV), 6-oxo-7-hydroxycholest-4-en-3β-yl acetate (CCLXXVI), 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII), 5-oxo-5,6-secocholest-3-en-6-oic acid (CCLXXXV) and 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVI)

(a) **With 1 mole equivalent of perbenzoic acid**

To a solution of 3β-acetoxycholest-4-en-6-one (CCLIV) (2 g) in chloroform (25 ml) was added a chloroform solution of perbenzoic acid (1 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate as catalyst and the reaction mixture left for 45 hours (The progress of the reaction was monitored by t.l.c.). The solvent was removed by distillation under reduced pressure and the residue extracted with ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), water, and dried over anhydrous Na_2SO_4. Removal of the solvent gave an oil (ca 2.0 g) which was chromatographed over silica gel (40 g)(each fraction of about 30 ml was collected). Elution with light petroleum-ether (12:1) gave 4-hydroxycholesta-2,4-dien-6-one (CCLXXIV), crystallized from light petroleum (80 mg) m.p. 95° (Found: C, 81.84; H, 10.40. $C_{27}H_{42}O_2$ requires C, 81.40; H, 10.55%). λ max (EtOH) 320 nm (ϵ 7700); ν max (Nujol) 3380br, 1660s, 1620 and 1100 cm^{-1}; δ ($CDCl_3$) 6.66m (C2-H), 6.0 dist.d (C3-H, major J 10 Hz), 2.7m (C7-H_2), 1.08, 0.91, 0.71 and 0.61 (5 methyl protons). Further elution with

light petroleum-ether (10:3) afforded the unreacted ketone (CCLIV) (630 mg), m.p.[98] and m.m.p. 110°.

Elution with light petroleum-ether (2:1) furnished 6-oxo-7α-hydroxycholest-4-en-3β-yl acetate (CCLXXVI), crystallized from light petroleum (85 mg), m.p. 165° (Found: C, 75.65; H, 10.11. $C_{29}H_{46}O_4$ requires C, 75.98; H, 10.04%). λ max (EtOH) 238 nm (ε 6200); νmax (Nujol) 3390br, 1740s, 1690s, 1645, 1235 and 1050 cm^{-1}; δ($CDCl_3$) 6.08 dist.d (C4-$\underline{H}$, major J 2 Hz), 5.37m (C3-$\alpha\underline{H}$, $w\frac{1}{2}$ 12 Hz), 3.87br,s (C7-$\beta\underline{H}$), 2.41br ($O\underline{H}^a$), 2.05s ($C\underline{H}_3COO$), 1.01, 0.81, 0.71 and 0.61 (5 methyl protons). Continued elution with the same solvent combination afforded 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII), crystallized from light-petroleum (300 mg), m.p. 190° (Found: C, 73.24; H, 9.81. $C_{29}H_{46}O_5$ requires C, 73.41; H, 9.70%). λ max (EtOH) 209 nm (ε 7800); νmax (Nujol) 3400-3200br, 2725w, 1740s, 1720s, 1690s, 1645 and 1240 cm^{-1}; δ ($CDCl_3$) 9.55 dist.d ($COO\underline{H}^a$ and $\underline{H}CO$; on D_2O shake J 5.5 Hz), 6.9 dist.d (C4-$\underline{H}$, major J 1.5 Hz), 5.3m (C3-$\alpha\underline{H}$, $w\frac{1}{2}$ 12 Hz), 2.6m (C8-$\beta\underline{H}$), 2.07s ($C\underline{H}_3COO$), 1.27, 0.9, 0.8 and 0.67 (5 methyl protons). Elution with light petroleum-ether (5:3) gave 5-oxo-5,6-secocholest-3-en-6-oic acid (CCLXXXV)[76] (450 mg) as a noncrystallizable oil (Found: C, 77.64; H, 10.43. Calcd. for $C_{27}H_{44}O_3$ C, 77.88; H, 10.57%). λ max (EtOH) 230 nm (ε 10000); νmax (Nujol) 3440-3200br, 1710s, 1680s and 1620 cm^{-1}; δ ($CDCl_3$) 8.77s ($COO\underline{H}^a$), 6.75m (C3-$\underline{H}$), 5.87d (C4-$\underline{H}$, J 10 Hz), 2.7m (C7-$\underline{H}_2$), 1.09, 0.88, 0.7 and 0.66 (5 methyl protons).

(b) With 2.5 mole equivalent of perbenzoic acid

The ketone (CCLIV)(2 g) on treatment with 2.5 mole equivalent of perbenzoic acid, under similar conditions as described before, followed by column chromatography gave (CCLXXVII) (270 mg), m.p. and m.m.p. 190°, (CCLXXXV) (500 mg) and 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVI) (from the solvent system light petroleum-ether, 5:4), crystallized from light petroleum (310 mg), m.p. 245° (Found: C, 70.84; H, 9.43. $C_{29}H_{46}O_6$ requires C, 71.02; H, 9.38%). λ max (EtOH) 212nm (ε 7550); ν max (Nujol) 3400-3200br, 1745s, 1710s, 1690s, 1625 and 1240 cm^{-1}; δ ($CDCl_3$) 7.4s, 7.35s (2 x $COO\underset{a}{H}$), 6.83 dist.d (C4-$\underline{H}$, major J 1.5 Hz), 5.41m (C3-αH, $W\frac{1}{2}$ 13 Hz), 2.6m (C8-$\beta\underline{H}$), 2.1s ($C\underline{H}_3COO$), 1.45, 0.91, 0.83 and 0.68 (5 methyl protons).

6-Oxocholesta-2,4-dien-4-yl Acetate (CCLXXV)

A mixture of 4-hydroxycholesta-3,4-dien-6-one (CCLXXIV) (40 mg), pyridine (0.6 ml) and acetic anhydride (0.4 ml) was allowed to stand at room temperature for 60 hours. The reaction mixture was worked up in the usual manner to provide the acetate (CCLXXV)(30 mg) as a noncrystallizable oil (Found: C, 80.0; H, 9.82. $C_{29}H_{44}O_3$ requires C, 79.69; H, 10.0%). λ max (EtOH) 315nm (ε 7208); ν max (Nujol) 1770s, 1685s, 1630, 1210, 1100 and 1050 cm^{-1}.

Methyl 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oate (CCLXXIX)

An ethereal solution of 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII)(350 mg) was treated with an excess of an ethereal solution of diazomethane in the cold and after 10 minutes the reaction mixture was worked up in the usual manner. Removal of the solvent gave (CCLXXIX), crystallized from light petroleum (310 mg), m.p. 150° (Found: C, 73.85; H, 9.80. $C_{30}H_{48}O_5$ requires C, 73.77; H, 9.83%). λ max (EtOH) 210 nm(ε 7750); νmax (Nujol) 2740w, 1745s, 1720s, 1645, 1240 and 1080 cm^{-1}; δ($CDCl_3$) 9.43d ($\underline{H}CO$, J 6 Hz), 6.58 dist.d (C4-$\underline{H}$, major J 1 Hz), 5.2m (C3-$\alpha\underline{H}$, $W\frac{1}{2}$ 11 Hz), 3.7s ($COOC\underline{H}_3$), 2.5m (C8-$\beta\underline{H}$), 2.0s ($C\underline{H}_3COO$), 1.23, 0.88, 0.80 and 0.65 (5 methyl protons).

Methyl 7-hydroxy-3β-acetoxy-6,7-secocholest-4-en-6-oate (CCLXXX)

Methyl 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oate (CCLXXIX)(200 mg) in methanol (30 ml) was treated with sodium borohydride (15 mg in 5 ml of methanol) for 10 minutes at room temperature. The reaction mixture was diluted with water and extracted with ether. The ethereal solution was washed with water and dried over anhydrous Na_2SO_4. Removal of the solvent afforded (CCLXXX) (180 mg) as an oil (Found: C, 73.34; H, 9.96. $C_{30}H_{50}O_5$ requires C, 73.47; H, 10.20%). λ max (EtOH) 210 nm (ε7200); νmax (Nujol) 3500br, 1740s, 1720s, 1635, 1230, 1190, 1065 and 1025 cm^{-1}; δ($CDCl_3$) 6.55 dist.d (C4-$\underline{H}$, major J 1 Hz)

5.33m (C3-$\underline{H}$, $W\frac{1}{2}$ 12 Hz), 3.93d (C7-$\underline{H}_2$, J 5.5 Hz), 3.72s ($COOC\underline{H}_3$), 2.09s ($C\underline{H}_3COO$), 1.7br ($O\underline{H}$), 1.21, 0.9, 0.8 and 0.65 (5 methyl protons).

Methyl 3β,7-diacetoxy-6,7-secocholest-4-en-6-oate (CCLXXXI)

A mixture of methyl 7-hydroxy 3β-acetoxy-6,7-secocholest-4-en-6-oate (CCLXXX)(70 mg), pyridine (0.6 ml) and acetic anhydride (0.3 ml) was allowed to stand at room temperature for 70 hours under anhydrous conditions. Usual work up of the reaction mixture gave (CCLXXXI) as an oil (60 mg)(Found: C, 72.04; H, 9.65. $C_{32}H_{52}O_8$ requires C, 72.18; H, 9.79%). λ max (EtOH) 212 nm(ε 8000); νmax (Nujol) 1740s, 1720s, 1635, 1235, 1190, 1060 and 1020 cm^{-1}; δ($CDCl_3$) 6.58 dist.d (C4-$\underline{H}$, major J 1 Hz), 5.33m (C3-$\underline{H}$, $W\frac{1}{2}$ 12 Hz), 4.15d (C7-$\underline{H}_2$, J 5 Hz), 3.76s ($COOC\underline{H}_3$), 2.05s (2 x $C\underline{H}_3COO$), 1.22, 0.9, 0.8 and 0.65 (5 methyl protons).

Jones' oxidation of (CCLXXX): Methyl 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oate (CCLXXIX)

Methyl 7-hydroxy-3β-acetoxy-6,7-secocholest-4-en-6-oate (CCLXXX)(40 mg) was dissolved in acetone and the solution cooled below 10° in ice-water bath. Jones' reagent (0.3 ml) was added to the solution with continuous shaking for 20 minutes. Water (10 ml) was added to it and resultant mixture was extracted with ether which after usual work up gave (CCLXXIX)(30 mg), m.p. and m.m.p. 150°

3β-Hydroxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXXII)

A solution of 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII)(250 mg) in 50 ml of methanolic NaOH (2%) was heated under reflux for 2 hour. The mixture was poured into water, acidified with HCl and extracted with ether. Usual work up of the ether solution gave (CCLXXXII), crystallized from light petroleum (220 mg), m.p. 195° (Found: C, 74.86; H, 10.20. $C_{27}H_{44}O_4$ requires C, 75.0; H, 10.02%). λ max (EtOH) 215nm (ε 7600); νmax (Nujol) 3500-3200br, 2730w, 1720s, 1695s, 1640 and 1065 cm^{-1}; δ($CDCl_3$) 9.46 dist.d (COO$\underset{a}{H}$ and $\underline{H}$CO; on D_2O shake J 5 Hz), 6.96dist,d (C4-$\underline{H}$, major J 1 Hz), 5.83br (O$\underset{a}{H}$), 4.35m (C3-$\alpha\underline{H}$, $W\frac{1}{2}$ 12 Hz), 2.5m (C8-$\beta\underline{H}$), 1.27, 0.9, 0.8 and 0.67 (5 methyl protons).

3,7-Dioxo-6,7-secocholest-4-en-6-oic acid (CCLXXXIII)

3β-Hydroxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXXII) (160 mg) was subjected to Jones' oxidation in the usual fashion which afforded (CCLXXXIII), crystallized from light petroleum (140 mg), m.p. 160° (Found: C, 75.44; H, 9.82. $C_{27}H_{42}O_4$ requires C, 75.35; H, 9.76%). λ max (EtOH) 238 nm (ε 11670); ν max (Nujol) 3400-3200br, 2735w, 1720s, 1700s, 1685s and 1615 cm^{-1}; δ ($CDCl_3$) 9.3d ($\underline{H}$CO, J 5 Hz), 7.99br,s (COO$\underset{a}{H}$), 6.66s (C4-$\underline{H}$), 2.5m (C8-$\beta\underline{H}$ and C2-$\underline{H}_2$), 1.4, 0.9, 0.81 and 0.7 (5 methyl protons).

Methyl 3,7-dioxo-6,7-secocholest-4-en-6-oate (CCLXXXIV)

3,7-Dioxo-6,7-secocholest-4-en-6-oic acid (CCLXXXIII) (85 mg) was treated with diazomethane in the usual manner to afford the methyl ester (CCLXXXIV), crystallized from light petroleum (70 mg), m.p. 125° (Found: C, 75.81; H, 9.82. $C_{28}H_{44}O_4$ requires C, 75.67; H, 9.91%). λmax (EtOH) 238nm (ε 11200); νmax (Nujol) 2740w, 1725s, 1710s, 1680s, 1620 and 1140 cm^{-1}; δ($CDCl_3$) 9.36d (HCO, J 5 Hz), 6.48s (C4-H), 3.81s ($COOCH_3$), 2.6m (C8-βH and C2-H_2), 1.48, 0.9, 0.8 and 0.65 (5 methyl protons).

Methyl 5-Oxo-5,6-secocholest-3-en-6-oate (CX)[77]

5-Oxo-5,6-secocholest-3-en-6-oic (CCLXXXV)(200 mg) was treated with an excess of an ethereal solution of diazomethane and after usual work up of the reaction mixture the methyl ester (CX)(185 mg) was obtained as an oil (Found: C, 77.75; H, 10.51. Calcd. for $C_{28}H_{46}O_3$ C, 77.90; H, 10.69%). λmax (EtOH) 230nm (ε9987); νmax (Nujol) 1735s, 1675s, 1615 and 1165 cm^{-1}; δ($CDCl_3$) 6.75m (C3-H), 5.8d (C4-H, J 10 Hz), 3.57s ($COOCH_3$), 2.8m (C7-H_2), 1.07, 0.88, 0.8 and 0.66 (5 methyl protons).

Dimethyl 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylate (CCLXXXVII)

3β-Acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVI)(100 mg) was treated with diazomethane in the usual

manner to furnish the diester (CCLXXXVII) (80 mg) as an oil (Found: C, 71.54; H, 9.35. $C_{30}H_{48}O_6$ requires C, 71.42; H, 9.52%). λmax (EtOH) 213nm (ϵ 7500); νmax (Nujol) 1740s, 1720s, 1640, 1240 and 1185 cm^{-1}; δ ($CDCl_3$) 6.63dist.d (C4-H, major J 1 Hz), 5.3m (C3-αH, $W\frac{1}{2}$ 12 Hz), 3.75s (C5-$COOCH_3$), 3.65s (C8-$COOCH_3$), 2.55m (C8-βH), 2.09s (CH_3-COO), 1.25, 0.9, 0.81 and 0.65 (5 methyl protons).

3β-Hydroxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVIII)

A solution of 3β-acetoxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVI)(250 mg) in 50 ml of methanolic NaOH (2%) was heated under reflux for 2 hours. Usual work up and removal of the solvent furnished (CCLXXXVIII), crystallized from light petroleum (200 mg), m.p. 225° (Found: C, 72.56; H, 9.74. $C_{27}H_{44}O_5$ requires C, 72.32; H, 9.82%). λmax (EtOH) 212 nm (ϵ 7450); νmax (Nujol) 3400-3100br, 1700s, 1695s, 1620 and 1080 cm^{-1}.

3-Oxo-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXIX)[78]

3β-Hydroxy-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXVIII)(150 mg) was subjected to Jones' oxidation in the usual manner and this afforded (CCLXXXIX), crystallized from light petroleum (130 mg), m.p. and m.m.p. 192° (Found: C, 72.50; H, 9.32. Calcd. for $C_{27}H_{42}O_5$ C, 72.64; H, 9.41%). λmax (EtOH) 235nm (ϵ 8920); νmax (Nujol) 3400-3100br, 1725s, 1700s, 1670s and 1610 cm^{-1}.

Dimethyl 3-Oxo-6,7-secocholest-4-ene-5,8-dicarboxylate (CCXC)

An ethereal solution of 3-oxo-6,7-secocholest-4-ene-5,8-dicarboxylic acid (CCLXXXIX)(80 mg) was treated with an excess of an ethereal solution of diazomethane to furnish (CCXC), crystallized from light petroleum (70 mg), m.p. and m.m.p. 140° (Found: C, 73.28; H, 9.74. Calcd. for $C_{29}H_{46}O_5$ C, 73.41; H, 9.70%). λ max (EtOH) 236nm (ε 9550); νmax (Nujol) 1735s, 1715s, 1675s, 1620, 1185 and 1175 cm^{-1}; δ ($CDCl_3$) 6.46s (C4-H), 3.81s (C5-$COOCH_3$), 3.66s (C8-$COOCH_3$), 2.9-2.4br (C8-βH and C2-H_2), 1.2, 0.9, 0.8 and 0.65 (5 methyl protons).

Reaction of methyl 7-hydroxy 3β-acetoxy-6,7-secocholest-4-en-6-oate (CCLXXX) with perbenzoic acid

A solution of (CCLXXX) (80 mg) in chloroform was treated with perbenzoic acid in chloroform (1 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate. The reaction mixture was left at room temperature for 48 hours and checked by t.l.c. from time to time. No change was noted, however, and the reaction mixture on usual work up provided the unchanged (CCLXXX) (70 mg).

Reaction of 6-oxo-7α-hydroxycholest-4-en-3β-yl acetate (CCLXXVI) with perbenzoic acid

The compound (CCLXXVI)(70 mg) was treated with a chloroform solution of perbenzoic acid (2.5 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate as catalyst. The progress of the reaction was monitored by t.l.c. and after 48 hours the reaction mixture was worked up in the usual manner. Column chromatography over silica gel of the crude product afforded (CCLXXVI) (30 mg), m.p. and m.m.p. 245°.

Reaction of 3β-acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII) with perbenzoic acid

To a chloroform solution of (CCLXXVII)(100 mg) was added a chloroform solution of perbenzoic acid (2.5 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate. T.l.c. of the reaction mixture at regular intervals showed no change and after a period of 48 hours, the reaction mixture was worked up in the usual fashion to afford the unreacted (CCLXXVII) (85 mg), m.p. and m.m.p. 190°.

Attempted oxidation of C7-oxo function in (CCLXXVII) with Jones' reagent

3β-Acetoxy-7-oxo-6,7-secocholest-4-en-6-oic acid (CCLXXVII) (100 mg) was dissolved in acetone and the solution cooled below

10° in ice-water bath. Jones' reagent (0.5 ml) was added to the solution with continuous shaking for 30 minutes. Water (25 ml) was added and the resultant mixture extracted with ether which on usual work gave the unreacted (CCLXXVII) (90 mg), m.p. and m.m.p. 190°.

3β,5,6β-Trihydroxy-5α-cholestane

A mixture of cholesterol (20 g) and formic acid (20 ml, 88%) was heated on a water bath at 70-80° for 5 minutes and then allowed to attain room temperature. Hydrogen peroxide (20 ml, 30%) was added to the mixture and it was kept at room temperature for 12 hours with occasional shaking. Boiling water (300 ml) was added with stirring and the reaction mixture allowed to attain room temperature when a white granular solid separated which was filtered under suction and air dried. The solid was dissolved in methanol (600 ml) and the solution heated with NaOH solution (20 ml, 25%) for 10 minutes on a steam bath. It was acidified with HCl and diluted with boiling water (300 ml). The triol obtained on cooling was collected by filtration under reduced pressure and recrystallized from methanol (17.5 g), m.p. 237-39° (reported[99] m.p. 237-39°).

5-Hydroxy-5α-cholestane-3,6-dione (CCXVIII)

A suspension of 3β,5,6β-trihydroxy-5α-cholestane (5 g) in acetone (200 ml) was cooled in an ice bath. Jones' reagent (15 ml) was added gradually with stirring over a period of 30 minutes. Water (200 ml) was added to the reaction mixture and the precipitate thus obtained was collected by filtration under suction. The crude product (CCXVIII) was recrystallized from methanol (3.2 g), m.p. 255° (reported[78] m.p. 232-33°).

Cholest-4-ene-3,6-dione (CCXXI)

A mixture of 5-hydroxy-5α-cholestane-3,6-dione (CCXVIII) (2 g), dioxan (140 ml), and conc. H_2SO_4 (2 ml) was heated under reflux for 1 hour. The solvent was removed under reduced pressure and the residue was diluted with water and extracted with ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the solvent provided the desired dione (CCXXI), which was recrystallized from light petroleum (1.5 g), m.p. 122-23° (reported[78] m.p. 122-23°). λmax (EtOH) 252 nm (ε 10300); νmax (Nujol) 1680s, 1615 and 1595 cm^{-1}; δ ($CDCl_3$) 6.16s (C4-H), 2.6mc (C2-H_2 and C7-H_2), 1.16 (C10-CH_3), 0.72 (C13-CH_3), 0.96, 0.9 and 0.84 (other methyl protons).

Baeyer-Villiger oxidation of cholest-4-ene-3,6-dione (CCXXI); 4-hydroxy-6-methoxycholesta-4,6-dien-3-one (CCXCV), 7α-hydroxy-cholest-4-ene-3,6-dione (CCXCII), 3-hydroxy-3,4-oxidocholest-4-en-6-one (CCXCVI), 5α,7aα-oxido-6-oxa-B-homocholestane-3,7-dione (CCXCVIII) and 5α,7aα-oxido-3,6-dioxa-A,B-bishomocholestane-4,7-dione (CCCIV)

(a) **With 1 mole equivalent of perbenzoic acid**

To a solution of cholest-4-ene-3,6-dione (CCXXI)(2 g) in chloroform (25 ml) was added a chloroform solution of perbenzoic acid (1 mole equivalent) and a few crystals of p-toluenesulphonic acid monohydrate as catalyst and the reaction mixture allowed to stand at room temperature for 60 hours. The solvent was removed by distillation under reduced pressure and the residue extracted with ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the desiccant and solvent provided an oil (ca 2.0 g) which was chromatographed over silica gel (40 g) (each fraction of about 30 ml was collected). Elution with light petroleum gave no product. Elution with light petroleum-ether (10:1) gave 4-hydroxy-6-methoxycholesta-4,6-dien-3-one (CCXCV), crystallized from light petroleum (70 mg), m.p. 100° (Found: C, 77.86; H, 10.51. $C_{28}H_{44}O_3$ requires C, 78.08; H, 10.48%). λ max (EtOH) 335 nm (ε 22700); ν max (Nujol) 3400br, 1650s, 1608, 1200, 1060 and 1030 cm^{-1}; δ ($CDCl_3$) 5.76d (C7-H, J 1 Hz), 3.71s ($COOCH_3$), 2.3m

(OH^{a} and C2-H$_2$), 1.1, 0.91, 0.81 and 0.73 (5 methyl protons). Further elution with light petroleum ether (10:3) led to a solid (500 mg), m.p. 85-90° (a very intimate mixture of CCXXI and CCXCVIII).

Elution with light petroleum-ether (2:1) afforded 7α-hydroxycholest-4-ene-3,6-dione (CCXCII), crystallized from light petroleum (400 mg), m.p. 165° (Found: C, 79.36; H, 10.18. $C_{27}H_{42}O_3$ requires C, 78.26; H, 10.19%). λ max (EtOH) 245 nm (ε 12216); ν max (Nujol) 3330br, 1700s, 1680s, 1625, 1225 and 1040 cm^{-1}; δ ($CDCl_3$) 6.1s (C4-H), 3.98br,s (C7-βH), 2.3m (OH^{a} and C2-H$_2$), 1.15, 0.9, 0.81 and 0.7 (5 methyl protons). Continued elution with chloroform-MeOH (20:1) furnished 3-hydroxy-3,4-oxidocholest-4-en-6-one (CCXCVI), crystallized from light petroleum (40 mg), m.p. 195° (Found: C, 78.31; H, 10.03. $C_{27}H_{42}O_3$ requires C, 78.26; H, 10.10%). λ max (EtOH) 275 nm (ε 6575); ν max (Nujol) 3420br, 1670s, 1620 and 1060 cm^{-1}; δ ($CDCl_3$) 3.08br,s (1p, H-C2-C3(OH)-O-C4), 2.3m (OH^{a} and C7-H$_2$), 1.2, 0.9, 0.81 and 0.66 (5 methyl protons).

(b) <u>With 2 mole equivalent of perbenzoic acid</u>

The ketone (CCXXI)(2 g) when treated with perbenzoic acid (3 mole equivalent) under similar reaction conditions for 8 days, gave after usual work up procedure, followed by column chromatography over silica gel, compounds (CCXCVI)(10 mg), m.p. and m.m.p. 195° (aforesaid solvent system i.e. chloroform-MeOH 20:1)

and 5α,7aα-oxido-6-oxa-B-homocholestane-3,7-dione (CCXCVIII) (from elution with light petroleum-ether 10:3), crystallised from light petroleum (430 mg), m.p. 115°; M^+ 430 ($C_{27}H_{42}O_4$) (Found: C, 75.30; H, 9.94. $C_{27}H_{42}O_4$ requires C, 75.34; H, 9.76%). ν_{max} (Nujol) 1798s, 1720s, 1180, 1140 and 920s cm^{-1}; δ ($CDCl_3$) 5.6s (C7a-$\underline{H}$), 2.9d (1p, J 15 Hz; gem coupling), 3.3d (1p, J 15 Hz; gem coupling, C4-$\underline{H}_2$), 1.01, 0.90, 0.80 and 0.70 (5 methyl protons).

(c) With 3 mole equivalent of perbenzoic acid

The ketone (CCXXI) (2 g) on treatment with perbenzoic acid (3 mole equivalent) under similar conditions for 8 days, followed by column chromatography over silica gel gave (CCXCVIII) (320 mg), m.p. and m.m.p. 115° and 5α,7aα-oxido-3,6-dioxa-A,B-bishomocholestane-4,7-dione (CCCIV) (from solvent system light petroleum-ether 5:4), crystallized from light petroleum (270 mg) m.p. 193°; M^+ 446 ($C_{27}H_{42}O_5$)(Found: C, 72.85; H, 9.47. $C_{27}H_{42}O_5$ requires C, 72.64; H, 9.41%). ν_{max} (Nujol) 1795s, 1735s, 1200, 1142 and 920s cm^{-1}; δ ($CDCl_3$) 5.6s (C7a-$\underline{H}$), 4.4t (C2-$\underline{H}_2$, J 5 Hz), 3.2d (1p, J 15 Hz; gem coupling), 2.6d (1p, J 15 Hz; gem coupling; C4a-$\underline{H}_2$), 1.05, 0.90, 0.80 and 0.70 (5 methyl protons).

6,7-Diacetoxycholesta-4,6-dien-3-one (CCXCIII)

A mixture of 7α-hydroxycholest-4-ene-3,6-dione (CCXCII) (100 mg) purified pyridine (0.3 ml) and distilled acetic anhydride

(0.2 ml) was heated on a steam bath for 15 hours under anhydrous condition. The resulting solution was poured into ice cold water and extracted with ether. The ethereal solution was washed with water, dil. HCl, water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Removal of the solvent provided (CCXCIII), crystallized from light petroleum (70 mg), m.p. 125° (Found: C, 74.28; H, 9.31. $C_{31}H_{46}O_5$ requires C, 74.49; H, 9.23%). λ max (EtOH) 320 nm (ϵ 21704); ν max (Nujol) 1760s, 1750s, 1680s, 1650, 1570, 1220, 1210 and 1020 cm^{-1}; δ ($CDCl_3$) 6.11d (C4-H, J 1.5 Hz; long range coupling with C2-proton), 2.35m (C2-H_2), 2.2s, 2.15s (2 x CH_3-COO), 1.2, 0.9, 0.8 and 0.7(5 methyl protons).

6-Acetoxycholesta-4,6-dien-3-one (CCXCIV)

A mixture of cholest-4-ene-3,6-dione (CCXXI)(260 mg), purified pyridine (0.4 ml) and distilled acetic anhydride (0.3 ml) was heated on a steam bath for 15 hours under anhydrous conditions. Usual work up and removal of the solvent provided (CCXCIV), crystallized from light petroleum (160 mg), m.p. 112° (Found: C, 80.68; H, 10.21. $C_{29}H_{44}O_3$ requires C, 79.10; H, 10.0%). λmax (EtOH) 293 nm (ϵ 23200); νmax (Nujol) 1770s, 1680s, 1640, 1590, 1210 and 1190 cm^{-1}; δ($CDCl_3$) 5.78d (J 1.5 Hz), 5.75s (C4-H and C7-H; unassigned), 2.4m (C2-H_2), 2.18s (CH_3COO), 1.18, 0.90, 0.81 and 0.76 (5 methyl protons).

3 —Acetoxy-3,4-oxidocholest-4-en-6-one (CCXCVII)

A mixture of 3-hydroxy-3,4-oxidocholest-4-en-6-one (CCXCVI) (120 mg), purified pyridine (0.3 ml) and distilled acetic anhydride (0.2 ml) was allowed to stand at room temperature for 72 hours under anhydrous condition. The usual work up procedure and removal of the solvent provided (CCXCVII), crystallized from light petroleum (95 mg), m.p. 145° (Found: C, 75.73; H, 9.89. $C_{29}H_{44}O_4$ requires C, 76.09; H, 9.65%). λ max (EtOH) 288 nm (ε 8491); ν max (Nujol) 1740s, 1690s, 1642, 1240, 1150, 1120 and 1020 cm^{-1}; $\delta(CDCl_3)$ 3.66br,s (1p, H-C2-C3(OAc)(O)C4), 2.4m (C7-H_2), 3.0s (CH_3COO), 1.26, 0.90, 0.81 and 0.66 (5 methyl protons).

Reaction of 7α-hydroxycholest-4-ene-3,6-dione (CCXCII) with perbenzoic acid

7α-Hydroxycholest-4-ene-3,6-dione (CCXCII)(100 mg) was subjected to perbenzoic acid (1.2 mole equivalent) oxidation under similar conditions as described before, and progress of the reaction was monitored by t.l.c. After 4 hours, the usual work up and removal of the solvent provided an oil which was chromatographed over silica gel (3 g) (each fraction of 5 ml was collected). Elution with light petroleum-ether (10:3) gave (CCXCVIII)(60 mg), m.p. and m.m.p. 115° (t.l.c. and i.r. identical with the earlier obtained CCXCVIII).

Reaction of 5α,7aα-oxido-6-oxa-B-homocholestane-3,7-dione (CCXCVIII) with perbenzoic acid

5α,7aα-Oxido-6-oxa-B-homocholestane-3,7-dione (CCXCVIII) (100 mg) was treated with perbenzoic acid (2 mole equivalent) under similar conditions and allowed to stand at room temperature for a week. The usual work up procedure and removal of the solvent provided an oil which was chromatographed over silica gel (2 g) (fractions of ~ 5 ml each were taken). Elution with light petroleum-ether (5:4) afforded (CCCIV) (25 mg), m.p. and m.m.p. 193° (t.l.c. and i.r. identical with the earlier obtained CCCIV).

PART - II

Preparation of hydrazoic acid solution

The hydrazoic acid solution was prepared according to Moural and Syhora[45]. Sodium azide (4 g) was dissolved in water (20 ml) and to this was added benzene (30 ml) at 0°. Sulphuric acid (4 ml) was then added dropwise with shaking over a period of 30 minutes at 0-5°; shaking was continued for an additional 30 minutes and the organic layer was separated, dried and filtered. This solution of hydrazoic acid (about 30 ml) was raised up to 50 ml by addition of benzene and was used in the reactions with ketones.

Reaction of 5α-cholestan-6-one (XXXIII) with an excess of hydrazoic acid: 6-Aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCVI) and 6-aza-B-homo-5α-cholestan-7-one (CCCIX)

To a cooled solution of hydrazoic acid in benzene prepared as above was added boron trifluoride etherate (1.5 ml, freshly distilled) and to this was added a solution of (XXXIII)(2 g) in benzene (25 ml) over a period of about 5 hours and the mixture was allowed to stand at room temperature for 30 hours. The benzene solution was washed with water, $NaHCO_3$ solution (5%), water and dried over anhydrous Na_2SO_4. Benzene was removed by distillation under reduced pressure and the residue (ca. 2.0 g)

thus obtained was chromatographed over silica gel (40 g) and fractions of about 30 ml were collected. Elution with benzene-chloroform (8:2) afforded (CCCVI), recrystallized from light petroleum-ether mixture (1.5 g), m.p. 169° (Found: C, 75.98; H, 10.85; N, 13.0. $C_{27}H_{46}N_4$ requires C, 76.05; H, 10.8; N, 13.14%). ν max (KBr) 1540, 1460 and 1380 cm^{-1}; δ ($CDCl_3$) 4.26dd (C5-αH, $J_{a,a}$ 10 Hz; $J_{a,e}$ 7 Hz), 3.21d (C7a-H, J 15 Hz), 0.43s (C13-CH_3), 0.9, 0.81 and 0.83 (remaining methyl protons). Further elution with benzene-chloroform (2:1) gave the lactam (CCCIX) (100 mg), m.p.^{62} and m.m.p. 172°.

Reaction of 3β-acetoxy-5α-cholestan-6-one (XXXIV) with an excess of hydrazoic acid: 6-Aza-B-homo-3α-cholestano[6,7-d]tetrazol-3β-yl acetate (CCCX)

The ketone (XXXIV)(2 g) was treated with hydrazoic acid-boron trifluoride etherate in the manner described for (XXXIII). The removal of benzene provided an oil which was crystallized from light petroleum-ether mixture to give (CCCX)(1.2 g), m.p. 185° (Found: C, 71.97; H, 10.0; N, 11.60. $C_{39}H_{48}N_2$ requires C, 71.90; H, 9.92; N, 11.68%). ν max (KBr) 1730, 1525, 1455, 1360 and 1240 cm^{-1}; δ ($CDCl_3$) 4.78br (C3-αH, $W\frac{1}{2}$ 14 Hz), 4.45dd (C5-αH, $J_{a,a}$ 14 Hz; $J_{a,e}$ 7 Hz), 3.4d (C7a-H, J 15 Hz), 2.06s (CH_3COO), 0.55s (C13-CH_3), 0.91, 0.83 and 0.65 (remaining methyl protons). The filtrate was stripped of the solvent and the residue

was chromatographed over silica gel (18 g) and fractions of about 20 ml were collected. Elution with benzene-ether (10:3) gave (CCCX)(500 mg), m.p. and m.m.p. 195°. Further elution with benzene-ether (5:2) afforded a compound, m.p. 170° (50 mg) which analysed for five nitrogen atoms but remained uncharacterized due to its small quantity.

6-Aza-B-homo-5α-cholestano[6,7-d]tetrazol-3β-ol (CCCXII)

A solution of tetrazole (CCCX)(500 mg) in 100ml methanolic NaOH (5%) was heated under reflux for 2 hours. The excess of methanol was removed under reduced pressure and the residue was diluted with cold water, acidified with HCl, filtered under suction, washed with water and air dried. The solid was recrystallized from chloroform-light petroleum mixture to give (CCCXII) (400 mg), m.p. 190° (Found: C, 73.26; H, 10.43; N, 12.7. $C_{27}H_{46}N_4O$ requires C, 73.35; H, 10.41; N, 12.67%). ν max (KBr) 3400, 1540, 1480 and 1390 cm^{-1}; δ ($CDCl_3$) 4.33dd (C5-$\underline{H}$, $J_{a,a}$ 12 Hz; $J_{a,e}$ 7 Hz), 3.75br (C3-$\underline{H}$, $w\frac{1}{2}$ 12 Hz), 3.39d (C7a-$\underline{H}$, J 15 Hz), 0.52s (C13-$C\underline{H}_3$), 0.91, 0.81 and 0.63 (remaining methyl protons).

Acylation of tetrazole (CCCXII)

A mixture of (CCCXII)(200 mg), purified pyridine (0.4 ml) and distilled acetic anhydride (0.3 ml) was heated on a steam bath

for 2 hours. The usual work up and removal of solvent gave (CCCX) (120 mg), m.p. and m.m.p. 185°.

Reaction of 3β-hydroxy-5α-cholestan-6-one (CCLII) with an excess of hydrazoic acid; 6-Aza-B-homo-5α-cholestano[6,7-d]tetrazol-3β-ol (CCCXII) and 3β-hydroxy-6-aza-B-homo-5α-cholestan-7-one (CCCXIII)

The treatment of ketone (CCLII)(2 g) with hydrazoic acid boron trifluoride etherate in the usual manner gave a residue (ca.2.0 g) which was chromatographed over silica gel (40 g) and fractions of about 30 ml were collected. Elution with chloroform provided tetrazole (CCCXII)(1.2 g), m.p. and m.m.p. 190°. Further elution with chloroform-methanol (20:1) gave (CCCXIII), m.p. 83 and m.m.p. 277-78°.

3β-Chloro-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCXIV)

Freshly purified thionyl chloride (0.5 ml) was added to tetrazole (CCCXII)(500 mg) at room temperature. A vigorous reaction ensued with the evolution of gaseous products. When the reaction slackened the mixture was gently heated at a temperature of 50-60° on a water bath for 1 hour and then poured onto crushed ice with stirring. The yellowish solid thus obtained was filtered under suction and air dried. The solid product was

chromatographed over silica gel (10 g) and fractions of about 20 ml were collected. Elution with benzene-ether (10:1) gave (CCCXIV), crystallized from light petroleum (30 mg), m.p. 195° (Found: C, 70.39; H, 9.8; N, 12.3. $C_{27}H_{45}N_4Cl$ requires C, 70.43; H, 9.78; N, 12.21%). ν max (KBr) 1340, 1470, 1380 and 730 cm^{-1}; δ($CDCl_3$) 4.66dist.dd (C5-$\underline{H}$, $J_{a,a}$ 10 Hz; $J_{a,e}$ 6 Hz and C3-$\underline{H}$), 3.46 (C7a-$\underline{H}$, J 15 Hz), 0.53 (C13-$C\underline{H}_3$), 0.93, 0.93 and 0.66 (remaining methyl protons).

Reaction of 3β-chloro-5α-cholestan-6-one (CCXLIX) with an excess of hydrazoic acid: 3β-Chloro-6-aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCXIV) and 3β-chloro-6-aza-B-homo-5α-cholestan-7-one (CCCXV)

The ketone (CCXLIX)(2 g) was treated with hydrazoic acid boron trifluoride etherate in the usual manner which provided a semi solid material (ca. 2.0 g). This was chromatographed over silica gel (40 g) and fractions of about 30 ml were taken. Elution with benzene gave the unchanged ketone (CCXLIX) (200 mg), m.p. and m.m.p. 128°. Further elution with benzene-ether (10:1) afforded the tetrazole (CCCXIV)(1.3 g), m.p. and m.m.p. 195°. Continued elution with the same solvent system gave the lactam (CCCXV)(80 mg), m.p.[85] and m.m.p. 150°.

Sodium-pentyl alcohol reduction of (CCCXIV); 6-Aza-B-homo-5α-cholestano[6,7-d]tetrazole (CCCVI)

The tetrazole (CCCXIV) (200 mg) was dissolved in warm pentyl alcohol (10 ml) and sodium metal (1.2 g) was added to the solution in small portions with intermittent heating during 30 minutes. The solution was kept warm for an additional period of 2 hours. The reaction mixture was poured into cold water and worked up in the usual manner followed by column chromatography over silica gel (5 g). Elution with benzene-chloroform (8:2) afforded (CCCVI)(80 mg), m.p. and m.m.p. 160°.

6-Aza-B-homo-3-oxo-5α-cholestano[6,7-d]tetrazole (CCCXVI)

The tetrazole (CCCXII)(500 mg) was dissolved in acetone and was cooled in ice bath. Jones' reagent (2 ml) was added gradually with stirring over a period of 30 minutes. Water (25 ml) was added to the reaction mixture and the precipitate thus obtained was taken in ether. The usual work up and removal of solvent gave (CCCXVI), recrystallized from light petroleum-ether mixture (400 mg), m.p. 205° (Found: C, 73.68; H, 10.1; N, 12.68. $C_{27}H_{44}N_6O$ requires C, 73.63; H, 10.0; N, 12.73%). ν_{max} (KBr) 1722, 1530, 1460 and 1360 cm^{-1}; δ($CDCl_3$) 4.66dd (C5-$\underline{H}$, $J_{a,a}$ 13 Hz; $J_{a,e}$ 6 Hz), 3.5m (C7a-$\underline{H}_2$), 0.68s (C13-$\underline{CH}_3$), 0.91, 0.81 and 0.73 (remaining methyl protons).

4,6-Diaza-A,B-bishomo-3-oxo-5α-cholestano[6,7-d]tetrazole (CCCXVII)

To a solution of (CCCXVI) (500 mg) and conc. H_2SO_4 (0.5 ml) in dry benzene (3.5 ml), sodium azide (80 mg) was added slowly with stirring at room temperature. A brisk reaction ensued and after 1 hour the reaction mixture was poured onto crushed ice. The benzene layer was separated and the aqueous layer extracted with chloroform. Usual work up of the organic extracts and the removal of the solvents gave (CCCXVII) which was crystallised from chloroform-light petroleum (300 mg), m.p. 260° (Found: C, 71.18; H, 9.92; N, 15.42. $C_{27}H_{45}N_5O$ requires C, 71.21; H, 9.89; N, 15.38%). ν max (KBr) 3340, 3200, 1690, 1640, 1540, 1470 and 1390 cm^{-1}; δ ($CDCl_3$) 7.0br (NH, exchangeable with D_2O), 4.66br (C5-αH), 4.06m (C4a-H_2), 3.41m (C7a-H_2), 0.45s (C13-CH_3), 0.91, 0.81 and 0.65 (remaining methyl protons).

3,3-Ethylenedioxy-6-aza-B-homo-3α-cholestano[6,7-d]tetrazole (CCCXX)

A mixture of ethylene glycol (40 ml) and sodium-dried benzene (300 ml) was heated in a Dean and Stark apparatus for 1 hour to remove the traces of water. The oxo-tetrazole (CCCXVI) (3 g) and p-toluenesulphonic acid monohydrate (ca. 80 mg) were added and the mixture was heated under reflux for 12 hours with

simultaneous removal of water. Saturated $NaHCO_3$ solution was then added to the reaction mixture once it attained the room temperature and the benzene layer was separated. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Removal of the solvent afforded (CCCXX) as an oil which was crystallized from acetone (2.6 g), m.p. 195° (Found: C, 71.82; H, 10.12; N, 11.51. $C_{29}H_{48}N_4O_2$ requires C, 71.60; H, 10.29; N, 11.52%). ν max (KBr) 1535, 1455, 1380, 1160, 1100 and 1030 cm^{-1}; $\delta(CDCl_3)$ 4.46dd (C5-$\underline{H}$, $J_{a,a}$ 12 Hz; $J_{a,e}$ 6 Hz), 3.96s (O-$C\underline{H}_2$-$C\underline{H}_2$-O), 3.42d (C7a-$\underline{H}$, J 15 Hz), 0.51s (C13-$C\underline{H}_3$), 0.9, 0.81 and 0.63 (remaining methyl protons).

PART - III

The mass spectra were measured on AEI MS-9 mass spectrometer at 70 eV using a direct insertion sample inlet system at a source temperature of about 250°. The accurate mass measurements were related to fragment ions of heptacosafluorotributylamine at a resolving power of 15,000.

The value (m/e) of the fragment ions from various ring B ϵ-lactones are tabulated below. The values in parentheses are the relative abundance (%) of the peaks with respect to base peak taken as 100%, and the compositions of fragment ions as determined by accurate mass measurement.

7-Oxa-B-homo-5α-cholestan-6-one (CCXLVIII)

M^+ 402 (81.2; $C_{27}H_{46}O_2$), m/e 387 (25.0), 384 (11.5), 373 (18.7), 372 (62.5; $C_{26}H_{44}O$), 335 (11.2), 334 (43.7), 319 (10.0), 318 (18.7), 317 (9.0), 305 (21.2), 304 (47.5), 290 (12.5), 289 (53.7), 263 (37.5), 262 (67.5), 261 (77.5), 260 (17.5), 249 (12.5), 248 (20.0), 247 (51.25), 246 (13.5), 235 (11.2), 234 (16.2), 233 (12.5), 232 (31.2), 219 (25.0), 218 (70.0), 179 (16.2), 178 (12.5), 177 (32.5), 175 (11.2), 165 (16.2), 163 (20.0), 161 (12.5), 152 (15.0), 151 (26.2), 150 (25.0), 149 (50.0), 148 (18.7), 147 (26.2), 141 (35.0),

140 (15.0), 137 (25.0), 136 (29.5), 135 (68.75), 133 (25.0), 126 (43.7), 124 (15.0), 123 (81.2), 122 (60.0), 121 (50.0), 119 (21.2), 111 (26.2), 109 (81.2), 108 (68.7), 107 (75.0), 106 (15.0), 105 (25.0), 97 (40.0), 96 (35.0), 95 (100), 94 (43.7), 93 (77.5), 91 (17.5), 83 (45.0), 82 (27.5), 81 (68.7), 80 (18.7), 79 (56.2), 71 (31.2), 69 (65.0), 68 (35.0), 67 (68.7), 57 (62.5), 55 (66.2), 43 (68.7), 41 (53.7).

6-Oxa-B-homo-5α-cholestan-7-one (XXXV)

M^+ 402 (8.2; $C_{27}H_{46}O_2$), m/e 387 (1.9), 384 (9.8), 374 (3.7; $C_{26}H_{46}O$), 360 (2.9), 359 (9.8), 356 (5.8), 341 (3.5), 333 (1.7), 331 (2.3), 320 (5.4), 319 (21.6), 318 (100; $C_{22}H_{38}O$), 317 (9.8), 306 (3.9), 303 (11.3), 289 (2.1), 262 (9.2), 247 (8.0), 178 (9.8), 177 (4.1), 167 (5.6), 164 (4.1), 163 (9.0), 161 (3.9), 153 (4.1), 152 (8.4), 149 (6.8), 147 (4.7), 136 (9.4), 135 (12.7), 133 (7.8), 123 (8.2), 122 (7.4), 121 (11.3), 119 (6.4), 112 (14.7), 110 (5.8), 109 (13.3), 108 (13.7), 107 (16.0), 105 (7.8), 97 (8.6), 96 (7.4), 95 (15.0), 94 (7.4), 93 (13.5), 91 (8.2), 83 (11.3), 82 (5.8), 81 (14.5), 79 (12.7), 71 (7.8), 69 (13.7) 68 (7.8), 67 (12.7), 57 (14.1), 55 (3.9), 53 (5.2), 43 (32.3), 41 (23.5).

3β-Chloro-7-oxa-B-homo-5α-cholestan-6-one (CCLXVIII)

M^+ 436/438 (40.0:14.2; $C_{27}H_{45}O_2Cl$), m/e 421/423 (10.0:3.8), 418/420 (4.2:1.4), 406/408 (54.2:20.0; $C_{26}H_{43}OCl$), 402 (5.7),

401 (25.7), 400 (27.1), 391/393 (2.8:1.4), 351/353 (25.7:8.5), 334 (11.4), 323/325 (17.1:5.7), 309 (7.1), 305 (10.0), 304 (7.1), 297 (11.4), 293/295 (14.2:7.1), 282 (12.8), 266/268 (25.7:10.0), 263 (18.5), 262 (85.7), 261 (100), 260 (30.0), 252/254 (61.4:21.4), 247 (31.4), 216 (14.2), 205 (10.0), 177 (22.8), 175 (10.0), 165 (12.8), 163 (17.1), 162 (12.8), 161 (11.4), 160 (35.7), 159 (22.8), 157 (51.4), 152 (35.7), 149 (62.8), 147 (21.4), 145 (14.2), 139 (18.5), 138 (20.0), 137 (15.7), 136 (25.7), 135 (90.0), 133 (25.7), 123 (48.5), 122 (57.1), 121 (60.0), 120 (12.8), 119 (24.2), 111 (21.5), 110 (12.8), 109 (78.5), 108 (80.0), 107 (80.0), 106 (18.5), 105 (31.4), 97 (22.8), 96 (24.2), 95 (97.1), 94 (51.4), 93 (82.8), 92 (12.8), 91 (34.2), 85 (14.2), 83 (38.5), 82 (35.7), 81 (95.7), 80 (20.0), 79 (62.8), 77 (20.0), 71 (38.5), 70 (17.1), 69 (71.4), 68 (40.0), 67 (64.2), 57 (78.5), 56 (17.1), 55 (97.1), 43 (35.7), 41 (57.1).

3β-Chloro-6-oxa-B-homo-5α-cholestan-7-one (CCXLIII)

M^+ 436/438 (14.4: 5.0; $C_{27}H_{45}O_2Cl$), m/e 421/423 (2.7:1.1), 418/420 (2.3:1.1), 408/410 (3.3:1.1), 402 (8.3), 401 (30.4), 400 (22.2), 372 (6.6), 319 (25.0), 318 (100; $C_{22}H_{38}O$), 317 (8.3), 306 (5.0), 281 (5.0), 263 (10.0), 262 (38.8), 247 (5.5), 193(5.5), 179 (5.5), 178 (6.1), 170 (6.1), 167 (9.4), 163 (5.5), 153 (10.0), 152 (13.3), 149 (7.2), 147 (5.5), 141 (5.5), 139 (5.5), 136 (9.4), 135 (10.5), 134 (10.5), 123 (7.7), 122 (7.2), 121 (13.3), 119 (7.7),

109 (16.6), 108 (27.7), 107 (18.8), 105 (8.8), 97 (6.1), 96 (5.5), 95 (25.5), 94 (16.1), 93 (23.8), 91 (9.8), 83 (12.7), 82 (7.7), 81 (23.3), 79 (13.6), 71 (8.8), 69 (16.3), 68 (5.5), 67 (16.1), 57 (17.7), 55 (27.7), 43 (35.0), 41 (17.7).

7-Oxa-3-homo-6-oxo-5α-β-sitostanyl acetate (CCLW)

M^+ 488 (3.0; $C_{31}H_{52}O_4$), m/e 473 (1.3), 459 (2.6), 458 (6.9; $C_{30}H_{50}O_3$), 430 (6.6), 429 (35.3), 428 (100), 417 (6.6), 416 (18.0), 415 (54.0), 400 (5.3), 399 (7.3), 386 (6.6), 385 (10.6), 370 (6.6), 362 (14.6), 348 (8.3), 333 (6.0), 332 (8.0), 319 (5.3), 315 (8.6), 290 (14.0), 289 (10.0), 288 (8.0), 287 (15.0), 277 (4.6),276 (15.0), 275 (12.0), 274 (6.6), 273 (6.0), 262 (6.0), 261 (12.0), 248 (16.6), 247 (12.0), 235 (5.3), 234 (15.0), 233 (8.0), 216 (6.6), 177 (9.3), 175 (6.0), 163 (8.0), 161 (6.6), 149 (23.3), 148 (6.6), 147 (13.3), 140 (7.3), 139 (21.3), 138 (27.3), 137 (8.6), 136 (7.3), 135 (24.6), 134 (6.6), 133 (15.3), 125 (6.0), 124 (18.6), 123 (18.6), 122(18.0), 121 (24.0), 120 (6.0), 119 (12.0), 111 (10.0), 110 (6.6), 109 (27.3), 108 (22.0), 107 (32.6), 106 (7.3), 105 (16.6), 97 (13.3), 96 (9.3), 95 (52.0), 94 (62.0), 93 (46.6), 91 (15.3), 85 (13.3), 84 (6.0), 83 (16.0), 82 (9.3), 81 (36.0), 80 (6.0), 79 (23.3), 77 (6.6), 71 (18.6), 69 (27.3), 68 (10.0), 67 (22.6), 57 (35.3), 55 (46.6), 43 (58.6), 41 (16.5).

6-Oxa-B-homo-7-oxo-5α-β-sitostanyl acetate (CCLVI)

M^+ 488 (1.8; $C_{31}H_{52}O_4$), m/e 473 (1.0), 470 (0.4), 460 (0.6), 446 (3.7), 445 (6.2), 431 (4.1), 429 (9.3), 428 (28.1), 413 (14.1), 410 (2.7), 401 (8.1), 400 (24.3), 399 (4.7), 395 (8.3), 382 (5.4), 368 (4.3), 359 (3.3), 348 (5.4), 347 (23.5), 346 (100; $C_{24}H_{42}O$), 345 (10.6), 331 (16.2), 291 (4.5), 290 (16.8), 287 (5.2), 275 (6.2), 261 (5.8), 260 (4.1), 259 (3.1), 247 (4.1), 245 (5.2), 191 (5.6), 178 (7.9), 163 (7.0), 161 (5.8), 252 (10.4), 149 (6.2), 147 (6.2), 136 (5.8), 135 (10.8), 134 (7.5), 133 (11.4), 123 (6.4), 122 (5.4), 121 (7.5), 120 (6.2), 119 (8.9), 111 (7.9), 110 (6.2), 109 (12.0), 108 (12.5), 107 (15.6), 105 (9.5), 97 (7.2), 95 (25.0), 94 (18.7).

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LIST OF PUBLICATIONS

1. Steroid D-Ring Lactones: A Reinvestigation.
   Journal of Organic Chemistry (In press).

2. Reaction of 3β-Acetoxycholest-4-en-6-one with Perbenzoic Acid.
   Australian Journal of Chemistry (In press).

3. Synthesis of 6-aza-B-homo-5α-cholestano[6,7-d] tetrazole and its derivatives.
   Indian Journal of Chemistry (In press).

4. MASS SPECTRAL STUDIES ON STEROIDAL COMPOUNDS - VIII: A Comparative Study of 6- and 7-Oxalactones.
   Organic Mass Spectrometry (Communicated).